EXPERIMENTAL DENTAL CARIES[1]

III. A System of Recording Corn Meal Caries in Rats

GERALD J. COX, MARY L. DODDS, SARA F. DIXON, and MARGARET C. MATUSCHAK

The Nutrition Fellowship of The Buhl Foundation, Mellon Institute, Pittsburgh, Pa.

We have used corn meal[2] caries in rats for the study of three phases of nutrition in its relation to dental caries, namely, "1) nutrition during the period of formation of the teeth, 2) foods which initiate caries, and 3) factors which affect the rate of progress of decay" (2).

Our first use of corn meal caries in rats was in an investigation of rate of decay as affected by foods. Our premise was that regardless of the origin of the lesions, if the corn meal ration was replaced by diets that do not initiate decay, any increase in the size of the cavities would be by a true carious process. In the course of such studies we observed a significantly lowered incidence of decay ("macroscopic" or "fracture" caries as described by others) in rats whose mothers had been on a ration with a high content of fat; we also found lesions (the opaque type described below) that resemble beginning human caries and could scarcely have originated in gross fracture of the teeth. These observations suggested that corn meal caries could be used for the study of dietary factors operative in the formation of teeth resistant to decay and that the findings would be applicable to man.

The results of our studies using corn meal caries in rats are not in conflict with well-authenticated facts of nutrition as related to human caries. For example, we have found that fermentable carbohydrates promote decay in rat molars. We have observed a lower incidence of caries in rats whose mothers had an adequate supply of fluorine during pregnancy and lactation. These examples and other observations

[1] Presented in part at the Fifteenth General Meeting of the International Association for Dental Research, Baltimore, Maryland, March 13, 1937 (*J. D. Res.*, **16**: 302, 1937).

[2] We have simplified the ration of Hoppert, Webber and Canniff (*J. D. Res.*, **12**: 161, 1932) using yellow corn meal 66, whole milk powder 30, alfalfa meal 3, sodium chloride 1.

lead us to believe that corn meal caries in rat molars can provide much information for the prevention of human dental caries by formation of caries-resistant teeth and for the reduction of rate of decay in existing caries. Furthermore, rat molars may be satisfactory for investigation of the pertinent factors in initiation of decay in susceptible teeth.

Based on our premise of independence of origin of cavities and their subsequent development after removal of caries-initiating factors, we developed a system of study of rat caries using cavities visible under 15 diameters magnification in teeth which were not sectioned. Our first attempts to record incidence and intensity of caries dealt with individual teeth. We soon found, however, that no comparisons could be made between teeth comparable numerically but decayed in different areas. We therefore worked out a procedure of recording incidence in areas empirically observed to be subject to corn meal caries. Later, when we had devised an efficient method of sectioning teeth (3), we were able to make an extensive study of rat molars following the system of scoring devised by Rosebury, Karshan, and Foley (8).

SCORING METHODS

Scoring of Occlusal Caries: We prefer to designate as "occlusal" cavities the lesions visible in unsectioned rat teeth after a ration containing corn meal. "Fissure" cavities are those lesions seen in ground sections, such as described by Rosebury, Karshan, and Foley (7, 8).

Occlusal cavities, as produced by corn meal in our experience, appear in 17 pairs of areas in the 12 rat molars. Our system of recording occlusal lesions (and also the fissure cavities) provides for 1) description, 2) location, 3) number, and 4) size. Description of the cavities is related to location and size, so that a record of a cavity of given size in a certain area indicates a definite type of cavity, as presented below.

1. In *CENTER* lesions the initial damage to the enamel occurs on the line of the anterior-posterior axis. The areas are in the occlusal edge of distal walls of sulci in the first and second lower molars.

2. *FUSED CUSP* cavities occur lingual to the anterior-posterior axis of the upper first and second molars at the occlusal edge of the mesial walls of the sulci at a line of cusp fusion. In the lower first and second molars they appear in shallow longitudinal buccal fissures in the occlusal surface of the major distal cusps.

3. *OPAQUE* lesions appear initially as white areas which have lost the normal translucency of the enamel. In later stages surface continuity is lost. The sites are at the buccal end of the floor of the mesial sulcus of the lower first molar and similarly in the main sulcus

of the third lower molar; also on the gingival line of the buccal-distal angle of the first lower molar and similarly on the buccal-mesial angle of the second lower molar.

4. *LATERAL CUSP* lesions appear as strippings of the enamel or gross fractures of the mesial-buccal minor cusps of the second and third lower molars. Also lesions appear in the sulcus lying between the minor mesial-lingual cusp of the second upper molar.

5. Cavitation of the third upper molars occurs so infrequently that description of this lesion is omitted. It is possible that some conditions which we have not yet encountered may lead to more frequent caries in these molars, so we have provided for recording decay if it does occur.

Fig. 1 shows on a punched card[3] the 12 molars of the rat. The upper molars are normally examined inverted and for convenience these sketches are transposed. The areas subject to decay, numbered and indicated by arrows in this illustration, are specifically located as described in the summary below. Areas up to and including 10 are in the maxillary molars; the remainder are in the teeth of the mandible.

Areas	*Type*	*Molar*	*Location in Tooth*
1, 2	Fused cusp	1st	Anterior wall, mesial sulcus
3, 4	Fused cusp	1st	Anterior wall, distal sulcus
5, 6	Lateral cusp	2nd	Posterior buccal wall, mesial-lingual minor cusp
7, 8	Fused cusp	2nd	Anterior wall, distal sulcus
9, 10	Uncertain	3rd	Not definite, probably main sulcus
11, 12	Opaque	1st	Floor of buccal end of mesial sulcus
13, 14	Center	1st	Occlusal edge, posterior wall, main sulcus
15, 16	Fused cusp	1st	Buccal, occlusal and posterior to main sulcus
17, 18	Opaque	1st	Buccal, proximal angle at gingival line
19, 20	Center	1st	Mesial wall. distal cusp
21, 22	Opaque	2nd	Mesial, buccal, proximal angle at gingival line
23, 24	Lateral cusp	2nd	Mesial, buccal minor cusp
25, 26	Center	2nd	Occlusal edge, posterior wall, main sulcus
27, 28	Fused cusp	2nd	Buccal, occlusal, posterior to main sulcus
29, 30	Center	2nd	Mesial wall, distal cusp
31, 32	Side cusp	3rd	Mesial, buccal minor cusp
33, 34	Opaque	3rd	Floor of buccal end of main sulcus

The sketches in *fig. 1* are provided with scores of 1, 2, and 3. The score appropriate to the cavity is encircled, a score of 1 being the first damage to the enamel, 2 indicates involvement of dentin, and 3 extensive cavitation, frequently being complete destruction of the area. If 2 or more adjacent areas have cavities which are merged, each area is given a score of its degree of cavitation. We have found it necessary to remove debris from cavities by means of dissecting needles

[3] The Keysort card of the McBee Company, Athens, Ohio.

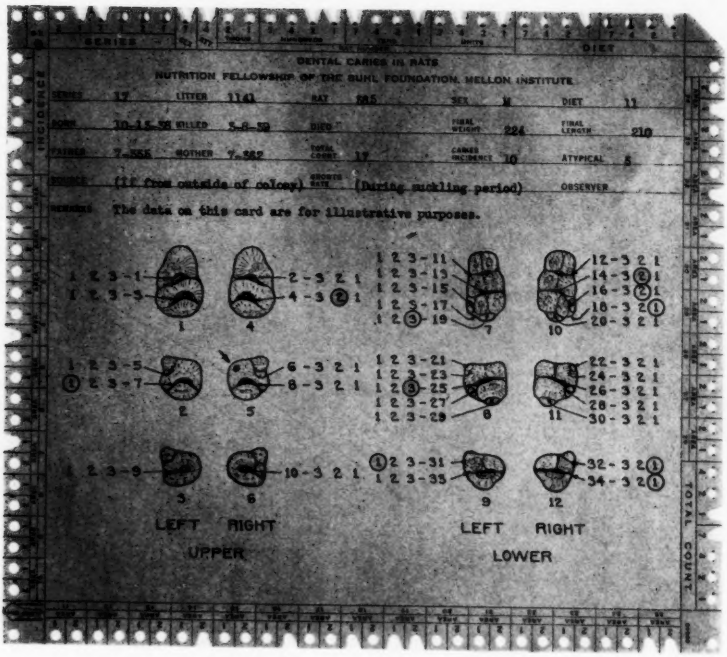


FIG. 1. Form for recording occlusal cavities

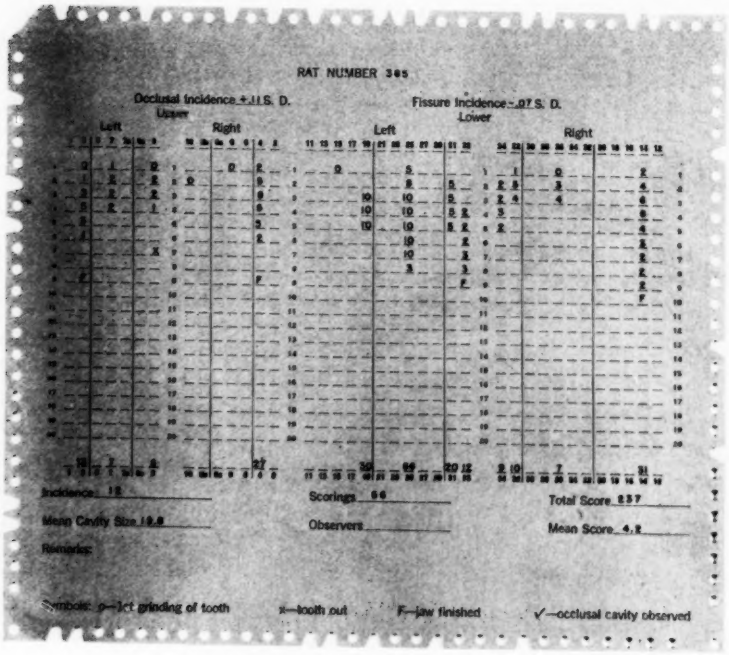


FIG. 2. Form for recording fissure cavities

(made from fine explorers) as we score, in order to make proper evaluation of the size of the cavities.

Scoring of Fissure Caries: Fig. 2 gives our record form for fissure caries. We have used the reverse side of the card, shown in *fig. 1*, thus keeping the records together and utilizing the punched card feature for sorting the data. We have given numbers to areas which may be susceptible to decay. These numbers are the same as for adjacent occlusal areas with the addition of 7a–10a, inclusive. Certain of these designated areas later proved to be identical with those recorded for occlusal areas and are therefore not used in fissure scoring.

The description of the cavities is that of Rosebury, Karshan, and Foley (8). In general, these cavities appear in their early stages as crescent-shaped areas along the dentino-enamel junction of *each* sulcus, except in areas 7a and 8a, as will be related. Cavities in these areas were not originally included in our occlusal scoring and hence were transferred to the fissure records when they appeared.

The following list contains the locations of the fissure cavities.

Areas		*Molar*	*Location in Tooth*
1, 2	Upper	1st	Mesial sulcus
3, 4		1st	Distal sulcus
5, 6		2nd	Sulcus distal to minor lateral cusp
7, 8		2nd	Main sulcus
7a, 8a		2nd	Distal cusp summit
9a, 10a		3rd	Sulcus distal to minor lateral cusp
9, 10		3rd	Main sulcus
11, 12	Lower	1st	Mesial sulcus
13, 14		1st	Distal sulcus
15, 16		1st	Identical with occlusal lesion
17, 18		1st	Identical with occlusal lesion
19, 20		1st	Mesial to distal minor cusp
21, 22		2nd	Identical with occlusal lesion
23, 24		2nd	Distal to minor cusp, mesial-buccal angle
25, 26		2nd	Main sulcus
27, 28		2nd	Identical with occlusal lesion
29, 30		2nd	Mesial to distal minor cusp
31, 32		3rd	Distal to minor cusp, mesial-buccal angle
33, 34		3rd	Main sulcus

The teeth are prepared, scored for occlusal caries, mounted, and ground in successive planes (3). As various features develop in successive sections, they are recorded for the respective areas in the

space opposite the number for the grind, the vertical column of numbers 1 to 20, inclusive. We record the beginning of grinding of any tooth with *0;* and if the tooth is ground away before completion of others, and the thin shell of remaining enamel drops out, we show its disappearance with *x*. Other symbols are presented in *fig. 2*.

The score of fissure caries is in the system of Rosebury, Karshan, and Foley (8). The numerical score for each carious area is recorded for each section and when the record is complete the scores are totaled, the *number* of cavities being counted as the incidence. Though we have provision on our record card for reduction of these values to other forms, so far we have used only the *incidence* in studies of prevention of decay and initiation of caries and the *total score* for individual areas for size comparison with cavities in litter mates in the study of arrest of caries.

STATISTICAL INTERPRETATION OF DATA

Our use of a punched card for recording dental caries in rats, especially the occlusal scorings, greatly facilitates study of the data. The examination of frequency of decay in any area, the association of decay in one area with lesions in any other area, or the elimination of any type of decay from the data are readily done. Likewise easy to perform are the routine analyses described below.

In order that our data may be comparable among all experimental groups, we have 1) begun feeding of the corn meal ration at weaning of the young rats at 21 days, 2) continued the ration for eight weeks, and, 3) if progress of caries is to be studied, used the non-caries producing diet for an additional 8 weeks. The standardization of time eliminates one important variable from a situation already too complex. If the time element is being studied, we have of course departed from these schedules.

Incidence of Caries: For the study of prevention of caries by dietary means during the period of formation of the teeth or of factors which initiate decay, we use as our criterion the mean number of cavities per animal, designated in *fig. 1* as "caries incidence." The mean incidence in an experimental group of rats is compared with that of a control group, using the standard error of the difference of the means as the unit of measurement.

We have found by the χ^2 test of Pearson (4) for a group of 63 animals of uniform dietary history that the distribution of the individual incidences of occlusal caries has a probability of 0.22 for a fit to the normal error function; for the fissure caries for a different group of 180 rats the probability was 0.90. Because it is known that the distribution of *means* tends more toward the normal than single observations, and, it is with the *means* that we are concerned, there is adequate justification for use of the integral tables of the normal error function in evaluating the data of both occlusal and fissure caries in rats.

By formula for difference of incidence of caries we evaluate t in

$$t = \frac{M_a - M_b}{\sqrt{\sigma^2_{M_a} + \sigma^2_{M_b}}}$$

in which M_a and M_b are the respective mean incidences and σ_{M_a} and σ_{M_b} their standard errors derived from the respective standard deviations, σ_a and σ_b, by division by n_a and n_b, in which n_a and n_b are the number of rats in series a and b, respectively. In general, if t exceeds 2, the difference of the means is regarded as significant. The odds against the chance occurrence of a given difference, as measured in terms of its standard error, can be found from tables of the normal distribution curve integral for the value of t.

In a series of 443 rats we found between the *scores* of occlusal and fissure caries a correlation coefficient of +0.64. The positive correlation suggests a common etiology of the two types of lesions. Regression equations were also derived but, since they suggest causal relationship between the lesions, they are omitted. But in each case the equations indicate that as the teeth are improved the occlusal caries disappears first. This is in agreement with some cases several years ago in which we discerned no occlusal caries but Rosebury (6) found fissure caries by his technique.

We have observed that the standard deviation of a single observation of incidence of decay in rats on the corn meal ration is about 4.7. If no preponderance of zero incidence occurs, such as in a series on good preventive pre-eruptive diets, the standard deviation is statistically constant and independent of the number of rats. The standard error of the mean, upon which our test of significance is based, is

inversely proportional to the number of rats. Small groups of rats can be used in studies of incidence of decay, but we have preferred to use enough animals so that a difference of 2 between the means would be significant, i.e., would yield a value of $t \geqq 2$. This condition is provided for a standard deviation of 4.7, if about 67 rats are used in each series.

We prefer to interpret separately our findings on initiation of decay in terms of occlusal and fissure caries parameters. We have found that tests of significance using the combined scores are less sensitive, probably because there is association between some fissure and occlusal cavities of the same area number.

Size of Cavities: For the study of alteration of rate of decay, either arrest or promotion of caries, we use rats weaned by mothers on a stock diet. The young are placed at 3 weeks age on the standard caries-producing diet for 8 weeks. Pairs of the same sex from the same litters are then put on 2 diets differing generally in a single constituent. After a further period of 8 weeks, all animals are killed and comparisons made of the size of cavities in identical areas.

The separation of the rats into 2 groups is by random selection to keep the mean weights statistically equal. It is therefore justifiable to assume that the 2 resulting groups have statistically equal numbers and sizes of cavities at the beginning of the experimental period. If the 2 rations do not differ in promoting the carious process, and neither initiates new cavities, statistical equivalence of number and size of cavities will continue throughout the experimental period. But, if the rations which do not initiate decay, differ in accelerating the carious process, the size of cavities of one group will increase and, at the end of the experiment, a greater number of cavities than that expected by chance will exceed the size of similarly located cavities in the litter mate. We test this deviation from that expected by chance by reference to the binomial distribution.

The formula for t used for comparison of rate of decay is

$$t = \frac{\mathrm{m} - \mathrm{b}}{\sqrt{\mathrm{npq}}}$$

in which b is the number of pairs of cavities in which the one on ration B is greater, m the mean of a and b, n the number of pairs of cavities,

p the probability that a will be greater than b, and q the probability that b will exceed a.

If we are without knowledge of the power of either of 2 rations A or B to promote decay, then the *a priori* probability, p, is 0.5 that a cavity in a molar of a rat on ration A is greater than a similarly located cavity in a rat on ration B. It follows that if a cavities are greater on ration A than similarly located cavities from ration B and b cavities are greater on ration B, we would expect a and b to be nearly equal, their most probable values being their mean, $\frac{a+b}{2}$, which is half the number of comparisons, designated by n.

The standard deviation of observations in the binomial distribution $\sqrt{npq}$, reduces to $\frac{\sqrt{a+b}}{2}$, when $n = a + b$ and $p = q = 0.5$. The equation for t then reduces to $t = \frac{a-b}{\sqrt{a+b}}$. If t, as calculated by this formula, exceeds 2 the odds are that some other factor than chance has caused the deviation. Odds against chance can be found for specific values of t from tables of the normal curve integral.

The data for a study of rate of decay can be considerably increased if lesions in teeth of one side of a rat can be compared for size with lesions in the opposite jaw of the litter mate. For such cases, which we term *contralateral* comparison, the *a priori* probability 0.5 as used for *homolateral* studies cannot be assigned, inasmuch as asymmetric susceptibility to decay may occur. But *a posteriori* probability can be determined by study of incidence and cavity size in the left and right teeth of a sufficient number of rats.

We have studied 1344 rats for the number and size of occlusal cavities in their left and right jaws. We found a greater tendency for initiation of decay in the molars in the right jaw for both males and females, but not a sufficient deviation from that within the normal chance expectation to be significant. We noted a total of 5243 occlusal cavities in the left molars and 5313 in the right.

We observed in all cases of pairs of cavities (including the "pair" in which no decay occurred in one area) 2604 cavities larger on the left and 2808 cavities larger on the right. Here is a significant trend, the odds against its chance occurrence being 177 to 1. Consequently

the probability of decay on the right being greater than on the left is $\frac{2808}{2604 + 2808}$, or 0.519; the probability of the left cavity being greater is 0.481. However, as the values are so nearly equal and, if the number of rats on each of the two diets is equalized to offset the bias, probabilities of 0.5 can be used with consequent simplification of the analysis. By similar reasoning the probabilities of 0.5 are used for comparison of fissure caries.

Effect of Inclusion of False Cavities: The variety of tooth injuries which we include as caries suggests that some types may not be caries in any sense. Until, however, the nature of such "lesions" is known we will record and include them in the analysis of the data. Our system of punched card records readily enables us to exclude any occlusal cavities if in the future they are found to be of non-carious nature.

If false cavities are included in the data of incidence, the random selection of the animals tends to distribute caries-like formations so that they are equally likely to appear in the experimental groups. The effect of their inclusion is to increase the mean in each group without affecting the difference of the means. The standard deviation, if affected, will be increased and such effect would be to increase the standard error of the difference of the means. Consequently, in the formula above for evaluation of the difference of means, t would be unaltered or diminished by false cavities.

Inclusion of false cavities in the data of size of cavities would add lesions not subject to decay, and, as they would be of equal likelihood of occurrence, their number may be considered as $2n'$. Half of them would increase a in the formula for t and half would increase b. The result would be

$$t = \frac{a + n' - (b + n')}{\sqrt{a + n' + b + n'}}$$

$$= \frac{a - b}{\sqrt{a + b + 2n'}}$$

The effect therefore of inclusion of false cavities in the data of either incidence or alteration of rate of decay is to render more conservative the conclusions drawn from evaluation of t.

DISCUSSION

Rosebury, Karshan, and Foley (7, 8) have expressed their observations of dental caries in rats in terms of a "caries index"; most other workers have reported only percentage of caries-free rats or teeth without reference to size or position of the lesions.

We early discarded the rat and the tooth as the unit in favor of carious areas in order to have a finer scale of measurement. With respect to the types of damaged enamel which we record as caries, we would find few caries-free animals and no distinction could be made between experiments. An animal with a single small cavity would be ranked equal with one with molars completely decayed away.

If the tooth is used as the unit much better grading of incidence of decay is obtained. But for the purpose of comparison of size of cavities between 2 animals for study of rate of decay, the lesions should exist in identical tissue, a condition best approximated by using identical sites. Thus, in our system, decay in area 1 is not to be compared with caries in area 3 in another animal, although these empirical sites are in the upper left first molar.

The caries index of Rosebury, Karshan, and Foley involves the use of number and size of cavities. Furthermore, it is derived from observation of a single cavity in part of the molars of each rat. Rosebury, Karshan, and Foley have used caries index in evaluating what we view as combined initiation and progress of decay for which we provide separate experiments interpreted, respectively, by 1) number and 2) size of cavities. Their caries index uses a restricted number of teeth and cavities which we feel entails loss of information.

We are aware that both number and size of cavities are indices of susceptibility to dental caries. Caries index includes both. Our system apparently uses only ***number*** of cavities as an index of susceptibility. But *size* of cavity is represented by counting a single large cavity as 2 or more, (1) depending on the number of areas involved. Thus a first lower molar completely decayed counts as 5 occlusal and 3 fissure cavities. If such a procedure were not adopted, an animal with several molars decayed away would show a smaller score than one with a number of small cavities in the same molars. We have conjectured that a tooth so susceptible to decay that such large cavities can develop in a short time must have been attacked in the

several areas involved. Thus our omission of size of cavity from the measure of susceptibility is partially compensated.

We have provided a "total count" (see *fig. 1*) which is the sum of the sizes of the cavities. We have hesitated to use total count, as it involves the summation of numbers that are non-linear; e.g., a cavity of size 2 is not twice the size of one with a score of 1. Furthermore, use of the parameters of total count has not given as fine a distinction between experiments as use of the parameters of incidence. We have used total count in a system of checking our punched card records for accuracy.

Miller (5) has recorded the number of lesions on a per hundred basis as a means of expressing caries incidence without reference to size.

Our system resembles that of Bödecker and Bödecker (1) for recording caries in man, in that it uses as its basis susceptible surfaces.

In our analysis of the data of incidence we have routinely prepared tables of the frequency and size of cavities in the specific areas. These tables serve as part of our check on the accuracy of our punched card record; but as the pertinent conclusions are drawn from mean incidence and standard error of the mean, we will not in future papers include tables of such distributions unless some significant differences in the sites of decay occur.

REFERENCES TO LITERATURE

1. Bödecker, C. F., and Bödecker, H. W. C., *D. Cosmos*, **73**: 707, 1931.
2. Cox, G. J., *D. Rays*, **13**: 8, 1937.
3. Cox, G. J., and Dixon, S. F., *J. D. Res.*, **18**: 153, 1939.
4. Fisher, R. A., *Statistical Methods for Research Workers* 5th ed., (London: Oliver and Boyd, 1934), p. 80 *et seq.*
5. Miller, B. F., *Proc. Soc. Exp. Biol. and Med.*, **39**: 389, 1938.
6. Rosebury, T., Personal Communication, Nov. 16, 1937.
7. Rosebury, T., Karshan, M., and Foley, G., *J. D. Res.*, **13**: 379, 1933.
8. Rosebury, T., Karshan, M., and Foley, G., *J. A. D. A.*, **21**: 1599, 1934.

EXPERIMENTAL DENTAL CARIES

IV. Fluorine and Its Relation to Dental Caries

GERALD J. COX, MARGARET C. MATUSCHAK, SARA F. DIXON, MARY L. DODDS, AND W. E. WALKER

The Nutrition Fellowship of The Buhl Foundation, Mellon Institute, Pittsburgh, Pa.

In the first of a series of papers on mottled enamel, Black in collaboration with McKay (6) indicated that there was a lower incidence of dental caries in mottled enamel areas than elsewhere. Bunting, Crowley, Hard, and Keller (7) found the percentage of children with dental caries in a mottled enamel district

"about the same as would be found in any other community, but although caries occurred in the mouths of most children, its extent and activity were remarkably limited. The great majority of cavities consisted of small pit and fissure lesions in the molars and seldom did caries extend beyond that stage. In this respect the behavior of dental caries in the mouths of these children is distinctly different to that which usually occurs."

McKay (25) in 1929 presented data showing definitely that mottled molars are less subject to dental caries than normal molars. The discovery of the causal relationship of fluorine to mottled enamel (8, 32) two years later led to numerous substantiating surveys of the fluorine content of water supplies and the resulting severity of mottled enamel, but the incidence of dental caries has not generally been reported. A notable exception is the observation by Arnim, Aberle, and Pitney (5) that of 1605 permanent incisors, 24 per cent having white spots in the enamel, none was carious. Masaki (26) and Erausquin (21) observed reduced incidence of dental caries in endemic mottled enamel areas. Ainsworth (1) described less caries in mottled enamel cases and of special interest found "12.9 per cent of deciduous teeth carious against 43.3 per cent in all districts." He learned of only one case in which deciduous teeth showed slight mottling.

Dean (15), from 1– previously unpublished data on dental caries in some of the cities surveyed for mottled enamel (16, 17, 18), from 2– his data of mottled enamel for South Dakota (19) correlated with

incidence of dental caries in the same areas (27), from 3– similar comparisons for 4 Colorado cities, and from 4– data on dental caries (27) and information from the Wisconsin State Board of Health on the fluorine content of the water supplies of 7 Wisconsin cities, concluded "that the severity of dental caries is, in general, lower in mottled enamel areas. . . ."

Dean, Jay, Arnold, McClure, and Elvove (20) compared the incidence of dental caries in 12, 13, and 14 year old children in Galesburg and Monmouth with that in Macomb and Quincy. These Illinois cities are comparable socially and meteorologically. Galesburg and Monmouth have water supplies with 1.7–1.8 p.p.m. of fluorine and 46.9 and 67.7 per cent of the children, respectively, have detectable mottled enamel. Macomb and Quincy have 0.2 p.p.m. of fluorine in drinking water and for the children 1.6 and zero per cent mottled enamel, respectively. The caries incidences are, in terms of carious teeth per 100 children: Galesburg, 201; Monmouth, 205; Macomb, 401; Quincy, 633. The respective percentages of caries-free children were 35.1, 35.1, 14.3, and 4.0. The distribution of children according to the number of *L. acidophilus* found in the saliva shows consistently more Galesburg children with the low numbers compared with Quincy. There was no significant difference in the amylolytic activities of the salivas from these latter 2 cities. The incidence of caries of interproximal surfaces of the 4 maxillary anterior teeth was extremely low in Galesburg and Monmouth, and mortality of the first permanent molars was about one-fourth that of Macomb and Quincy. The authors were convinced that water supplies affect dental caries incidence, but could not conclude that the higher fluoride content of the waters of Galesburg and Monmouth accounted for the better resistance to dental caries in these cities as compared with Macomb and Quincy because of other differences in the mineral composition of the water supplies.

Evidence of an entirely different kind for a beneficent rôle of fluorine was provided by Armstrong and Brekhus (2, 4), who reported the mean fluorine content of sound and carious human enamel as 0.0111 and 0.0069 per cent, respectively. They suggested a causal relationship.

Miller (28) inhibited caries development in rats by either 250 p.p.m. of sodium fluoride or 500 p.p.m. of calcium fluoride in a caries-produc-

ing ration. Miller's conditions, however, were entirely different from those concerned with the formation of caries-resistant teeth.

Sharpless and McCollum (31) observed no evidence of abnormality in the teeth of rats raised on a low fluorine diet.

The present authors, on evidence described in this paper, first suggested a beneficent rôle of fluorine in the formation of caries-resistant teeth in the published abstracts of the annual reports of the director of Mellon Institute (33) and elsewhere (10, 11, 22).

TABLE I

Milligrams per 4 grams of salt mixture

	SALT MIXTURE	
	Osborne and Mendel	Harris
	mg.	mg.
Sodium	137	136
Potassium	737	294
Calcium	496	273
Magnesium	64	58
Iron	12	24
Manganese	0.265	None
Aluminum	0.013	None
Phosphorus	310	409
Sulfur	27.7	76
Chlorine	478	113
Fluorine	(1.03)	None
Iodine	0.144	None
Ca/P	1.6	0.67

EXPERIMENTAL PROCEDURES

A ration of sucrose[1] 66, casein[2] 20, "Crisco" 10, and salt mixture 4[3] (See Table I) was used as the basis of the studies, except for diets 11–3 and 11–4.

The number of milligrams of each element contributed by 4 grams of each salt mixture to 100 grams of food is shown in Table I.

Merck's reagent sodium fluoride was added to the salt mixtures to provide the number of parts per million set forth in Table II. The basis of the addition was the 10.3 p.p.m. fluorine contributed by the sodium fluoride of the regular Osborne and Mendel salt mixture (30).

[1] Commercial refined cane sugar.

[2] Vitamin-free product of the Casein Company of America.

[3] Harris salt was purchased from The Harris Laboratories, Inc. The Osborne and Mendel salt (30) was made from C.P. reagents without the addition of sodium fluoride.

The data of Table I are based on the salts as added rather than by analysis.

Diets 11–3 and 11–4 were yellow corn meal 66, whole milk powder ("Klim", unirradiated) 30, alfalfa meal, powdered to pass 60-mesh sieve, 3, and C. P. sodium chloride 1.

During pregnancy, the animals on all diets except 11–3 and 11–4 received a daily supplement of 1 gram of fresh beef liver, 0.5 gram dried yeast, 0.5 gram of alfalfa, and 0.1 drop of Haliver oil with viosterol. They were given the same supplement during the lactation period with an addition of 1 gram each of yeast and alfalfa. The animals on diets 11–3 and 11–4 received distilled water *ad libitum*; the others were supplied with tap water containing 0.3 p.p.m. fluorine as shown by analysis.

The litters were reduced to 8 after three days and to 6 on the seventh day, preferably to 3 males and 3 females. The young were weaned at 21 days and placed on a diet of the same composition as diet 11–3, except ordinary sodium chloride was used.

All young were killed after 8 weeks on the corn meal caries-producing diet and the teeth were examined for occlusal and fissure caries.

TABLE II

Diets of rats during pregnancy and lactation and incidence of caries in their offspring

Diet Number	8–56	8–115	8–120	10–2	10–3	10–6	10–3+ 10–6	11–3	11–4
Number of Offspring	63	33	52	66	62	65	127	40	62
Salt Mixture[a]									
Harris	4	4							
Osborne and Mendel[b]			4	4	4	4			
Added Fluorine, p.p.m.	0	41.2	0	0	10.3	41.2		0	20.6
Occlusal Caries									
Mean Incidence	10.13	5.97	8.67	12.15	9.87	10.37	10.15	12.80	12.79
Standard Deviation	7.19	5.07	3.66	4.51	3.88	4.96	4.4	4.38	4.77
Fissure Caries									
Mean Incidence	16.79	11.76	16.77	16.98	16.71	15.84	16.27	19.00	19.77
Standard Deviation	3.46	5.12	3.45	3.95	3.56	4.55	4.12	3.12	3.13

[a] gm. per 100 gm. food.
[b] fluorine free.

RESULTS

The data relating to dental caries are shown in Table II. Table III (12) presents the interpretation by statistical analysis.

The studies using diets 8–56, 8–115, and 8–120 were completed in December, 1936, with respect to occlusal caries, the fissure caries scoring being done only recently. As the difference between diets 8–56 and 8–115 is solely the addition of sodium fluoride to the latter, and the chances are 1000 to 1 against the difference of caries incidence being due to chance, it may safely be concluded that the 41.6 p.p.m. additional fluorine has led to the greater caries resistance of the teeth.

The amount of sodium added is clearly negligible. It was on the basis of this experiment that we suggested (33) that fluorine may play a part in the formation of caries-resistant teeth.

We had purchased the Harris salt mixture as the Osborne and Mendel salt. But analysis during the course of the experimentation showed it to have only about one-fourth the expected fluorine. Correspond-

TABLE III

Odds that the occurrence of differences of mean incidence of caries is not due to chance

DIETS AND ADDED FLUORINE	CHANCES TO ONE	
	Occlusal Caries	Fissure Caries
8–56 vs. 8–115 0 41.2 p.p.m.	1000	2,500,000
8–56 vs. 8–120 0 0	5.3	—
8–115 vs. 8–120 41.2 p.p.m. 0	120	1,400,000
10–2 vs. 10–3 0 10.3 p.p.m.	480	0.47
10–2 vs. 10–6 0 41.2 p.p.m.	31	7
10–3 vs. 10–6 10.3 p.p.m. 41.2 p.p.m.	0.92	3.4
10–2 vs. 10–3 + 10–6 0	300	3.1
11–3 vs. 11–4 0 20.6 p.p.m.	—	3.4

ence with the manufacturer revealed that it was made by an older formula of Osborne and Mendel and contained no added fluorine. Therefore diet 8–120, containing no added fluorine, provided us with a salt mixture of entirely different composition (See Table I). The caries incidence between the diets with Osborne and Mendel salt mixture and the Harris salt does not differ significantly, but the

incidence on diet 8–120 is significantly different from that on diet 8–115. Thus this situation is somewhat analogous to that encountered by Dean, Jay, Arnold, McClure, and Elvove (20) as they could not conclude that fluorine was the factor responsible for difference in caries incidence between Galesburg and Quincy children because of the widely different composition of the mineral content of the drinking waters of these cities. Our experimental results are proof that fluorine alone plays a part in reducing caries incidence by its presence during the formation of the teeth.

The data of *fissure* caries on diets 8–56, 8–115, and 8–120 substantiate our conclusions based on *occlusal* caries, but the odds are far higher against pure chance having produced the caries distribution.

Diets 10–3 and 10–6 yielded rats with significantly greater resistance to *occlusal* caries than control diet 10–2. But *fissure* caries incidence was not significantly different. As diets 10–3 and 10–6 did not show a significant difference in occlusal caries, the data from these two diets were combined for comparison with the control ration. Again the difference is not to be ascribed to chance in the case of the occlusal caries; fissure caries incidence, though less for the diets with added fluorine, is still not significantly different from the control.

The control diets discussed were originally compounded for general studies on dental caries and materials were not specifically chosen for low fluorine content. We have not had satisfactory analyses of the control ration for fluorine and are in agreement with Armstrong (3), Dahle (14), and McClure (24) that the value of analyses for fluorine on materials which must be ashed is questionable. Considering that possibly a ration of natural materials might be used as a low fluorine diet, we have employed the corn meal caries-producing ration as the breeding diet in diets 11–3 and 11–4. According to Churchill, Bridges, and Rowley (9) and also McClure (24), this ration should be low in fluorine.

The breeders for the experimental rats were derived from animals fed diet 11–3 from the beginning of pregnancy and were raised on diet 11–3. Thus all the mothers for both 11–3 and 11–4 animals had lived all their lives on a low fluorine ration.

The addition of sodium fluoride equivalent to 20.6 p.p.m. of fluorine did not significantly affect the caries susceptibility of the young. In

fact, fissure incidence was increased in the average but only as would be expected by chance once in four trials. The incidence of both occlusal and fissure caries is the highest that we have observed on any ration, suggesting that some deficiency existed in the corn meal diet that obscured the protective action of the fluoride. The incidence on diet 8–115 is the lowest we have observed from a casein-sucrose type ration. Furthermore, in the preparation of the breeders for the experimental animals a deficit of fluorine may have been created in the mothers that was not corrected by the relatively short time feeding of fluoride from the beginning of pregnancy so that fluorine might be passed to the young. We have preliminary data from another type of experimentation that indicate that the beneficent action of fluorine occurs early in tooth life.

In a preceding experiment we have observed a reduction in caries incidence in young derived from mothers who had been fed a supplement of "XXX liquor" during pregnancy and lactation. As "XXX liquor," a trade waste of the milk industry, is sufficiently concentrated to be saturated with fluoride from the original milk, it is reasonable to credit the improvement to fluorine. Analyses, though of questionable precision, have revealed sufficient fluorine to be effective.

In studies on arrest of caries we have used many rats raised on rations containing commercial casein. Non-seasonal variation in caries incidence has occurred in these animals. The statement of Hodge, Luce-Clausen, and Brown (23) that commercial caseins may contain comparatively large amounts of fluorine suggests that the variable incidence may be ascribed to the variation in fluorine content of successive lots of commercial caseins used in our rations, and in confirmation we have observed that the incisors of rats raised concurrently on diets with the commercial casein show evidence of fluorosis.

DISCUSSION

Our evidence that fluorine aids in the formation of caries-resistant teeth, linked with the findings of Armstrong and Brekhus (4) and of Dean, Jay, Arnold, McClure, and Elvove (20), shows that a very great reduction of the incidence of human caries can be obtained by supplying in food and water an optimum amount of fluorine during

tooth formation. Concordant evidence from 3 different approaches, with no adverse data, should be such sufficient proof of the value of fluorine in the prevention of dental caries that means of control of this element in the whole dietary of children should be undertaken. Control of the fluorine content of community water supplies, in most cases by addition of fluorides, provides an attractive means of mass reduction in dental caries, but prophylactic measures through other media, such as bottled waters, milk supply or the judicious use of fluoride-containing medicinals, are feasible.

Regulation of fluorine should be directed at an optimum intake of the element. In particular, the fluorine content of the water supply can be varied seasonally to compensate for varying water consumption. Climatic differences will make it necessary for each locality to find its own standards for addition of fluorides to the water supply. The economics of the required water treatment will also be local problems.

As brought out in this report, the rat is a satisfactory animal for the study of the relation of fluorine to dental caries. We have recently (13) produced mottled enamel in the *molars* of rats and thus provided a means of experimental study of the interrelations of fluorine to dental caries and mottled enamel in a single species. The lowering of incidence of dental caries in rats by fluorine in the formation of teeth, linked with the observations of Dean, Jay, Arnold, McClure, and Elvove (20), indicates that corn meal caries in rats is true caries.

Present evidence on fluorine and dental caries is sufficient to require that all future studies of dental caries must consider the influence of fluorine and that past investigations be scanned for the possible contribution of this element. For example, the survey of Mills (29) on the relation of dental caries incidence to latitude and drainage basins can be interpreted in terms of fluorine.

McKay (25) has used mottled enamel as a proof that structure has nothing to do with resistance to dental caries. We predict that a structure resulting from lack of fluorine will be found which will differ from the "normal" and that therefore the effect of fluorine is one of modification of structure to a more caries-resistant form. On the other hand, the data of Miller (28), though derived from experiments with very high concentrations of fluorine, suggest that the action of

fluorine may be through bactericidal or bacteristatic action. A still further possibility is that fluorine exerts its effects prior to calcification and that the higher fluorine content of sound enamel, as observed by Armstrong and Brekhus (4), is only a sequela of the continuing higher supply of fluorine.

Whatever is the mechanism of the relative prevention of dental caries by fluorine, whether the resistance is structural, chemical, organic or combinations of these factors, it seems evident that caries resistance can be built into enamel.

REFERENCES TO LITERATURE

1. Ainsworth, N. J., *Brit. D. J.*, **55:** 233, 1933.
2. Armstrong, W. D., *Proc. Am. Soc. Biol. Chem., J. Biol. Chem.*, **119:** V, 1937.
3. *Idem*, Private Communication, April 19, 1939.
4. Armstrong, W. D., and Brekhus, P. J., *J. D. Res.*, **16:** 309, 1937; **17:** 393, 1938.
5. Arnim, S. S., Aberle, S. D., and Pitney, E. H., *J. A. D. A.*, **24:** 478, 1937.
6. Black, G. V., (in collaboration with McKay, F. S.) *D. Cosmos*, **58:** 129, 1916.
7. Bunting, R. W., Crowley, M., Hard, D. G. and Keller, M., *D. Cosmos*, **70:** 1002, 1928.
8. Churchill, H. V., *Ind. Eng. Chem., Ind. Ed.*, **23:** 996, 1931.
9. *Idem*, Bridges, R. W. and Rowley, R. J., *Ind. Eng. Chem., Anal. Ed.*, **9:** 222, 1937.
10. Cox, G. J., *D. Rays*, **13:** 8, 1937.
11. *Idem*, in *Dental Caries*, Advisory Committee on Dental Caries, A. D. A. (New York, 1939), p. 56.
12. Cox, G. J., Dodds, M. L., Dixon, S. F., and Matuschak, M. C., *J. D. Res.*, **18:** 469, 1939.
13. Cox, G. J., Matuschak, M. C., Dixon, S. F., and Walker, W. E., *Science*, **90:** 83, 1939.
14. Dahle, D., *J. Assoc. Official Agr. Chem.*, **22:** 338, 1939.
15. Dean, H. T., *Public Health Reports*, **53:** 1443, 1938.
16. Dean, H. T., and Elvove, E., *Public Health Reports*, **50:** 1719, 1935.
17. *Idem*, *Am. J. Pub. Health*, **26:** 567, 1936.
18. *Idem*, *Public Health Reports*, **52:** 1249, 1937.
19. Dean, H. T., Elvove, E., and Poston, R. F., *Public Health Reports*, **54:** 212, 1939.
20. Dean, H. T., Jay, P., Arnold, F. A., McClure, F. J., and Elvove, E., *Public Health Reports*, **54:** 862, 1939.
21. Erausquin, R., *Rev. Odont. Buenos Aires*, **23:** 296, 1935.
22. Hamor, W. A., *Science*, **87:** 381, 1938; **89:** 344, 1939.
23. Hodge, H. C., Luce-Clausen, E. M., and Brown, E. F., *J. Nutrition*, **17:** 333, 1939.
24. McClure, F. J., *Ind. Eng. Chem., Anal. Ed.*, **11:** 171, 1939.
25. McKay, F. S., *D. Cosmos*, **71:** 747, 1929.
26. Masaki, T., *Shikwa Gakuho*, **36:** Oct. 1931.
27. Messner, C. T., Gafafer, W. M., Cady, F. C., and Dean, H. T., *Public Health Bulletin* No. 226, U. S. Public Health Service, Washington, D. C., May 1936.
28. Miller, B. F., *Proc. Soc. Exp. Biol. and Med.*, **39:** 389, 1938.

29. MILLS, C. A., *J. D. Res.*, **16**: 417, 1937.
30. OSBORNE, T. B., AND MENDEL, L. B., *J. Biol. Chem.*, **32**: 369, 1917.
31. SHARPLESS, G. R., AND MCCOLLUM, E. V., *J. Nutrition*, **6**: 163, 1933.
32. SMITH, M. C., LANTZ, E. M., AND SMITH, H. V., *U. of Ariz. Tech. Bull.* No. 32: 253, 1931.
33. WEIDLEIN, E. R., *Ind. Eng. Chem., News Ed.*, **15**: 147, 1937; **16**: 228, 1938; **17**: 241, 1939.

A STUDY OF THE RELATIVE TOXICITY OF SODIUM FLUORIDE IN A DRY DIET, IN MILK, AND IN WATER

CECILIA SCHUCK, PH.D.

Department of Foods and Nutrition, Purdue University, Lafayette, Indiana

There is evidence that the toxicity of fluorides may be influenced by the medium in which they are ingested. In studies on the albino rat, Dean, Sebrell, Breux, and Elvove (1), observed a more toxic effect with a given concentration of sodium fluoride in the drinking water than with the same concentration in the diet. They were at a loss to explain this finding, but considered that the result might be due to differences in total quantity ingested, to a more complete absorption, to a more rapid rate of absorption, or to other factors.

Marcovitch, Shuey and Stanley (2) found higher concentrations of fluorine in the teeth of rats receiving fluorides in the drinking water than in those on similar levels of fluorine ingested as cryolite in the food. Later Marcovitch and Stanley (3) reported that rats receiving 4 p.p.m. of fluorine as sodium fluoride in the drinking water retained nearly twice as much of the element as rats receiving 4 p.p.m. of fluorine as cryolite in the diet. Striations occurred in the teeth with 4 p.p.m. of fluorine as sodium fluoride in the drinking water, but no striations were produced at the level of 4 p.p.m. of fluorine incorporated as cryolite in the diet. They attributed these results in part to the greater consumption of fluorine in the drinking water but concluded that solubility was also a factor. Lawrenz, Mitchell, and Ruth (4) found that with equal doses of fluorine there was a greater deposit of fluorine in bone, teeth, and soft tissue when administered in water than when administered in food, the explanation suggested being a difference in absorbability from the intestinal tract.

Since milk may in some instances affect significantly the fluorine intake, particularly of children, the toxicity of fluorine in milk is of concern in connection with the problem of mottled teeth. The Agricultural Experiment Station of the University of Wisconsin (5) has

reported studies in which it was found that sodium fluoride was seven to ten times more toxic when fed in milk than when fed in a grain ration. Investigations leading to a comparison of the relative toxicity of fluorides ingested in milk and in water have not been reported so far as is known to the author.

The purpose of this study was to compare the toxicity of sodium fluoride in milk with that of like concentrations in a dry diet and in water.

EXPERIMENTAL

Groups of albino rats twenty-four to twenty-six days of age and weighing forty to fifty grams were placed on the following diets:

Sherman's Diet B and distilled water.

Sherman's Diet B with 0.05 per cent sodium fluoride incorporated and distilled water.

Mineralized evaporated* milk (iron and copper salts added) and distilled water.

Sherman's Diet B and distilled water with the addition of sodium fluoride to give a 0.05 per cent concentration.

Mineralized evaporated milk containing 0.05 per cent sodium fluoride and distilled water.

The toxic effects of 0.05 per cent concentration of sodium fluoride in water was so great that only one of the first group of 6 rats survived the experimental period, the other five dying in 5 to 9 days. The experiment was then repeated with samples of water containing, in addition to the 0.05 per cent sodium fluoride, magnesium carbonate (0.125 per cent) in one case and sodium carbonate (0.7 per cent) in another case. Seven young rats were placed upon the high magnesium, high fluoride water and five on the high sodium, high fluoride water. The marked toxicity of the 0.05 per cent level of sodium fluoride in the drinking water was again evident, with little or no change as the result of the presence of the magnesium or sodium carbonate. All of the latter groups and all but two of the former died in from 4 to 14 days. The two in the former group surviving beyond that period lived 3 weeks and 5 days and 5 weeks and 1 day respectively.

A record was kept of the food intake and weight increment of the rats on the control diets (Sherman's diet B and mineralized evaporated milk) and on the diets containing sodium fluoride for a period of 4 weeks. The sodium fluoride intake for the period was estimated from the amounts of the sodium fluoride containing diets consumed. A summary of the results of the study is found in Table I.

A comparison of the effects of like concentrations of sodium fluoride in the dry diet and in the drinking water confirms the earlier findings. At the 0.05 per cent level the effects of like concentrations of sodium fluoride in the dry diet, in milk and in water indicate an increasing

* Evaporated milk was used because of the difficulty encountered in getting a sufficiently high nutritive intake with the unprocessed fluid milk. The development of diarrheas with the latter also produced complications.

toxicity from the first to the last. On the fluoride containing dry diet the growth rate was approximately 22 per cent less than on the control dry diet, but the fluoride containing milk diet resulted in a growth rate which was over 60 per cent less than that of the control milk diet. The voluntary food intake was decreased in both cases, with a slightly smaller decrease in the case of the milk diets. The rats receiving sodium fluoride in the milk as well as those on the sodium fluoride containing dry diet were able to survive the ill effects, while most of the rats on the fluoride containing water succumbed in from a few

TABLE I

Average food and sodium fluoride intake and gain in weight of rats on control and sodium fluoride containing diets for a period of 4 weeks

TYPE OF DIET	NUMBER AND SEX OF RATS	AVERAGE FOOD INTAKE	AVERAGE Na F INTAKE (GMS.)	AVERAGE GAIN IN WEIGHT (GMS.)		
				Males	Females	Average
Sherman's Diet B and distilled water	2 M 3 F	346 g.	—	76	69	72.5
Sherman's Diet B with 0.05 per cent Na F incorporated and distilled water	6 M 2 F	214 g.	0.107	56	57	56.5
Mineralized evaporated milk and distilled water	4 F	433 cc.	—	—	65	65.0
Mineralized evaporated milk containing 0.05 per cent Na F and distilled water	4 M	308 cc.	0.159	25	24	24.5
Sherman's Diet B and distilled water containing 0.05 per cent Na F	5 M 13 F	All but 2 died in less than 4 weeks, the average survival period being 8.6 days				

days to two weeks of time. The last group also showed the earliest signs of dental fluorosis, the teeth appearing white and chalky in rats of this group surviving beyond one week. In the other two groups two weeks was the minimal length of time for the appearance of outward dental signs, and the teeth of most of the rats on the fluoride containing dry diet showed no outward effects under four weeks.

Since most of the rats receiving sodium fluoride in the drinking water survived for only a short period and since an accurate record of the water intake was not obtained, an estimate of the actual amount

of sodium fluoride ingested by rats in these groups was not made. A comparison of the estimated intake of sodium fluoride in the milk and in the dry diet shows the former to be higher, and to this might be attributed in part the greater growth depression in the rats on the sodium fluoride containing milk diet. But in a previous study (6) there was a lesser effect on growth with an even greater estimated intake of sodium fluoride in dry diets. In Table II a comparison is made between estimated amounts of sodium fluoride ingested and gain in weight for a 4 weeks' period on the milk diet used in this study and on previously reported dry diets with 0.1 per cent sodium fluoride added.

TABLE II

Average sodium fluoride intake and weight gains of rats on milk containing 0.05 per cent and on dry diets containing 0.1 per cent sodium fluoride for a period of 4 weeks

TYPE OF DIET	NUMBER AND SEX OF RATS	AVERAGE Na F INTAKE (GMS.)	AVERAGE GAIN IN WEIGHT (GMS.)		
			Males	Females	Average
Control milk diet	4 F	—	—	65	65.0
Milk containing 0.05 per cent Na F	4 M 4 F	0.159	25	24	24.5
Control dry diet	2 M 2 F	—	98	68	83.0
Dry diet containing 0.1 per cent Na F	2 M 6 F	0.165	60	37	48.5

The results indicate that factors other than the amount ingested have rendered the sodium fluoride in the milk more toxic than that incorporated in the dry diets. The most important of these is probably increased solubility resulting in more rapid and more complete absorption. The findings here are significant in relation to the possibility that the fluoride content of milk produced in certain areas may be sufficient to play a part in the incidence of mottled teeth, particularly when the milk is consumed in amounts recommended for children. The extent, however, to which fluorides are transferred, through the milk is not known, although there appears to be some evidence (7 and 8) that transference may occur. The author (9) failed to obtain gross evidence of fluorosis (no analysis of tissues was made) when young rats were fed solely on milk shipped from a region where the

reported fluoride content of the water supply was 4 to 5 parts per million. It should be noted, however, that the minimum threshold of toxicity is very much lower for the human than for the rat (10 and 11).

More information is needed on the fluoride content of milk produced and used in regions where dental fluorosis is endemic. The high toxicity of sodium fluoride in water suggests a higher toxicity for a given concentration in unprocessed milk with its full water content than in evaporated milk. The need for further study here is also indicated.

SUMMARY

A study has been made of the effects of feeding albino rats like concentrations of sodium fluoride incorporated in a dry diet, in evaporated milk, and in water.

The results indicate an increasing toxicity from the first to the last. The toxicity of a 0.05 per cent concentration in water was so great that most of the rats died in from a few days to two weeks of time. The rats were able to survive on the fluoride-containing milk diet, but the growth rate was markedly depressed. There was a lesser depression of the growth rate with 0.05 per cent sodium fluoride incorporated in the dry diet.

The actual amount of sodium fluoride ingested in the milk diet was somewhat higher than in the dry diet, but apparently this does not account altogether for the difference in toxicity, since in a previous study a fluoride intake in a dry diet exceeding that in the milk affected growth less adversely.

Dental signs of fluorosis occurred in all three groups but appeared earliest in the few surviving rats receiving the sodium fluoride in the water.

The findings with respect to the toxicity of sodium fluoride in milk may have significance in relation to the possibility that the fluoride content of milk produced in certain regions may be sufficient to play a part in the occurrence of dental fluorosis in young children for whom milk constitutes the major part of the diet.

REFERENCES TO THE LITERATURE

1. Dean, H. T., Sebrell, W. H., Breaux, R. P. and Elvove, E., *Pub. Health Rep.*, No. 49, 1075, 1934.

2. MARCOVITCH, S., SCHUEY, G. A. AND STANLEY, W. W., *Cryolite Spray Residues and Human Health, Tenn. Agr. Exp. Sta. Bul.* 162, 1937.
3. MARCOVITCH, S. AND STANLEY, W. W., *J. Nutrition*, **16:** 173, 1938.
4. LAWRENZ, M., MITCHELL, H. H. AND RUTH, W. A., *J. Nutrition*, **18:** 127, 1939.
5. EVANS, R. J., PHILLIPS, P. H. AND HART, E. B., *Wis. Agr. Exp. Sta. Bul.* No. 439, 18, 1937.
6. SCHUCK, C., *J. D. Res.*, **17:** 387, 1938.
7. MURRAY, M. M., *J. Physiol.*, **87:** 388, 1936.
8. EVANS, R. J., *J. Dairy Sci.*, **21:** 81, 1938.
9. SCHUCK, C., Unpublished data.
10. SMITH, M. C. AND LANTS, E. M., *Experimental Production of Mottled Enamel, Ariz. Agr. Exp. Sta. Tech. Bul.* No. 45, 1933.
11. SMITH, M. C., *Amer. J. Pub. Health*, **24:** 696, 1935.

THE OCCURRENCE OF LACTOBACILLI AND OTHER ACIDOGENIC ORGANISMS IN THE SALIVAS OF SELECTED CARIES-FREE CHILDREN

MARSHALL L. SNYDER[1]

From the Hygienic Laboratory, University of Michigan, Ann Arbor, Michigan

In his monograph of 1889 Miller (1) outlined the now generally accepted view that bacteria in the presence of retained carbohydrate in protected areas of the teeth produce sufficient acid to cause decalcification of the enamel or dental caries. Since that time practically every acid-producing organism of the mouth has been accused at one time or another. However, after Kligler (2) showed the increased presence of *B. acidophilus* in the mouths of individuals with caries, a matter which was confirmed and extended by Rodriguez (3), the bulk of evidence has implicated the lactobacilli. At the Dental School of the University of Michigan an extensive program of research on dental caries is in progress. The Michigan group (4–11) and others have concluded that a lactobacillus, which they specifically call *Lactobacillus acidophilus*, is the organism to be associated with tooth decay. This taxonomical specificity is vigorously denied by Rettger and his associates (12–16). In addition, there are workers who have either been unable to confirm the correlation of lactobacilli with dental caries or insist that other organisms must be considered because of results with *in vitro* studies.

As part of the program at Michigan the author, working independently, was asked to examine the laboratory methods of the dental group; that is, first, to test the method for estimating the number of lactobacilli in the saliva; second, to determine whether or not lactobacilli were normal members of the mouth flora; and third, to correlate the cultural results with clinical findings at the beginning, middle, and end of a study on selected caries-free children. This paper covers the methods used to culture the specimens of saliva from these chil-

[1] Supported by a grant from the Horace H. Rackham School of Graduate Studies.

dren, the tabulation of organisms isolated on a selective medium, and an attempt to analyze the data in terms of the clinical findings. Although the material presented is insufficient to incriminate a species as the bacterial agent in the disease, the data do support the contention that lactobacilli are the bacteria responsible for caries.

Of the acidogenic organisms commonly found in the mouth the streptococci especially received attention from Goadby (17), Clark (18), Baumgartner (19), Kantorowicz (20), Hartzell and Henrici (21), Anderson and Rettger (22), Tunnicliff and Hammond (23), and others. However, since Kligler (2) in 1915 pointed out the increased presence of *B. acidophilus* in the mouths of people with caries, the lactobacilli have been more seriously considered primarily because of their physiological activity; that is, the growth of these forms on carbohydrate media is characterized by the production of large quantities of acid. Furthermore, the lactobacilli not only endure hydrogen-ion concentrations detrimental to the growth of most bacteria but they also multiply in this environment. From the data presented by Rodriguez (3), McIntosh, James and Lazarus-Barlow (23), Bunting et al. (4–11), Jay (24), Rosebury (25), Enright, Friesell and Trescher (26) and others, it is apparent that lactobacilli are generally present in the mouths of individuals with caries and are absent from those with no caries or arrested lesions.

On the other hand, Tucker (27) could find no apparent correlation between the presence of lactobacilli found on tooth scrapings and the dental caries observed in the respective subjects. In 1933, Johnston, Kaake and Agnew (28) stated that *L. acidophilus* was a member of the normal flora of the mouth in rats and man. In regard to man this statement reflects an impression held by many bacteriologists but for which there is little information in the reviews by Kligler (2), Nizzle (29), and the Cruickshanks (30). However, in 1936 Johnston et al. (31) reversed their earlier conclusions and were able to show a definite correlation between the presence of *L. acidophilus* and dental caries. Yet Anderson and Rettger (16) in 1937 could find lactobacilli in only 12 per cent of 65 patients with caries, and felt that other acidogenic organisms, particularly the streptococci, should not be disregarded. On the basis of examination of the dentin and pulps of 12 extracted teeth and 2 salivas from caries-free people, Tunnicliff and Hammond

(22) concluded that streptococci were the invaders in dental caries no matter what the predisposing factors.

METHODS

For the quantitative estimation of the numbers of lactobacilli in the saliva the method of Hadley (32) was closely followed. The specimens were obtained by having the individuals chew sterile wax tablets for 3 minutes and collect the saliva in sterile test tubes during that period. However, instead of diluting the specimens of saliva to 10 c.c. as recommended, measured quantities of saliva were smeared with sterile bent glass rods over acid tomato peptone agar plates, and results estimated in terms of lactobacilli per c.c. of the sample. In addition a portion of the specimen was inoculated into acid glucose broth for a qualitative test. The broth cultures were examined at 48 hours for the presence or absence of pleomorphic Gram-positive rods. On the plates after 96 hours incubation the colonies of lactobacilli appeared as variable but distinct morphological types either alone or associated with colonies of yeasts, streptococci, staphylococci, and sarcines. Those specimens of saliva which showed no lactobacilli on plates or in broth were considered to be negative. On the positive plates the characteristic colonies were examined and counted for the necessary calculations. The occurrence of some negative plates and positive broths was noted, and attributed to the failure of a few lactobacilli to grow on solid media.

Careful examination of each step of this technique showed it to give satisfactory and consistent results. The only place where one is likely to encounter difficulty is in the recognition and enumeration of the colonies of lactobacilli. The total count of the lactobacilli is not only influenced by the marked variation in the colony types, but also by all the objections to selected plate counts; namely, number of viable organisms in the saliva, number growing on selective media, colony representing growth from one or more germs, other types of bacteria, etc. Hence, by tacit admission the method is an estimation of the numbers of lactobacilli in the saliva, and while quantitative in one sense it is primarily relative. Its value lies in successive studies of individuals rather than in a single specimen of saliva; that is, the periodic recording whether lactobacilli are or are not present, sporadically present, or in low or high numbers gives more information than exact figures, especially in view of the statistical significance of errors in counts where 100,000 lactobacilli per c.c. of saliva or more are recorded.

In the present study all the types described by Hadley, Bunting and Delves (9) were seen. The colonies varied from the perfectly smooth white to the matted rough gray type. Microscopically, the Gram-

positive rods of these colonies appeared as forms scarcely to be distinguished from streptococci to the pleomorphic rods similar to those seen in a milk culture of *L. acidophilus*. Subculturing to acid glucose broth gave heavy flocculent growth; and litmus milk was acidified and coagulated. These studies were not sufficiently extensive to identify the oral lactobacillus as a species; hence, only the generic term "lactobacillus" is used.

SOURCES OF MATERIAL

Specimens of saliva were obtained from two selected groups of children. One group found to be free from caries by careful dental examination[2] was from an institution in Maumee, Ohio, where the children have been under observation for nearly a decade by the Michigan group. For the present study the salivas were collected in sterile test tubes under the supervision of a trained member of the school at irregular intervals for 18 months. This material was obtained in the morning and shipped to Ann Arbor where it was cultured in the evening. Cultures were made by smearing 0.4 c.c. saliva over the surface of tomato-peptone agar plates (for the one child with a high lactobacillus count 0.1 c.c. saliva was used after the first test), and placing 0.6 c.c. saliva in acid glucose broth for the qualitative test.

The other group was from two of the Ann Arbor public schools. Here the salivas were obtained by the author at intervals over the 18 month period and the samples plated upon return to the laboratory. The first cultures showed some high lactobacillus counts and others were negative. Therefore, 0.1 c.c. saliva was used for the individuals with numerous lactobacilli and 0.4 c.c. for those subjects with negative or low counts. The amount of saliva used varied, however, from time to time dependent upon the previous determination. For the qualitative tests 0.6–0.9 c.c. of the samples of saliva were placed in acid glucose broth.

RESULTS

The numbers of lactobacilli in the salivas of the two selected groups as estimated by the method outlined with a report on the presence or absence of caries at the end of the study is given in Table 1. The

[2] The author is indebted to Drs. Bunting, Jay, Hard and McCracken for the dental examinations at Maumee and Drs. Hard and Jay for the clinical work in Ann Arbor.

TABLE I

Numbers of lactobacilli in specimens of saliva from selected caries-free children cultured irregularly over an eighteen month period

GROUP	NUMBER	NUMBER OF CULTURES											DENTAL CONDITION AT END OF STUDY
		1	2	3	4	5	6	7	8	9	10	11	
Maumee	1	0	0	0	0	0	0	0	0	0			Negative 6/1/37—8 months
	2	375	0	0	0	0	0	0	0				Went home
	3	0	0	0	0	0	0	0	0	0	0		Negative
	4	6,000	5,600	9,300	8,400	15,000	10,800	120,000	2,400		12,000		Negative
	5	0	0	0	0	0	0	0	0	0	0		Negative
	6	0	0	0	0	0	0	0	0	0	0		Negative
	7	0	0	0	0	0	0						Went home
	8	0	0	0	0	0	0	0	0	0	0		One small extension
	9	0	0	0	0	0	0	0	0	0	0		Doubtful—negative
	10	0	0	0	0	0	0	0	0	0	0		Negative
	11	0	0	0	0	0	0	0	0	0	0		Negative
	12	0	0	0	0	0	0	0	0	0	0		Negative
	13	0	0	0	0	0	150	0	0	0	0		Negative
	14	0	0	0	0	0	0	0	0	0	0		Negative
	15	0	0	0	0	0	0	0	0	0	0		Negative
	16	0	0	0	0	0	0	0	0	0			Negative 6/1/37—8 months
	17	0	0	0	0	0	0	0	0				Went home
Ann Arbor	18	48,000	47,000	102,000	18,000	84,000			4,500				No information
	19	60,000	7,500	2,400	0	0	19,000	0	0	0	0	0	4 cavities, 2 pits, 2 fillings
	20	750	7,500	4,800	7,250	4,000	6,000	0	1,500		x	20	3 cavities
	21	0		0	0	0	0			0	0	0	Doubtful—negative
	22	9,300	29,000	30,000		21,000	22,000	210	125	0	0	0	Negative
	23	7,500		90,000		1,500	1,500	15,000	6,000	15,000	3,000	1,200	14 cavities
	24	100,000		600	120,000		900	0	100	1,800	60		Caries? 6/1/37, definite caries 6/1/38
	25	5,700	600	0	7,500		0	0	0		10,500	0	Doubtful—negative
	26	0		0	0	0	0	0	0	0	0		Negative
	27	0		0	0	0	0	0	0	0	0	0	Doubtful—negative
	28	0		0	0	0	0			0	0	0	Doubtful—negative
	29		100	14		0	0	0	30	0	0	0	Doubtful—negative
	30		75	375	450	0	6,300	0	1,200?	0	0	0	Negative

TABLE I—*Concluded*

GROUP	NUMBER	NUMBER OF CULTURES											DENTAL CONDITION AT END OF STUDY
		1	2	3	4	5	6	7	8	9	10	11	
Ann Arbor —*Con.*	31		31,500	0	0		120,000			1,500	800		2 small cavities
	32		45		4,500	7,500	900	0	1,200	4,500			Negative
	33		21,000	16,000	36,000	30,000	2,300	1,200	6,000	6,000	5,400		Doubtful—negative
	34		675	0	0	0		25	0	4,600	60,000		Small amount of caries
	35		0	0	0	0	0		0	0	0		Doubtful—negative
	36		0	0	0	150	0	150	0	0	0		Doubtful—negative
	37		0	0	0			0	0	0			Negative 6/1/37—8 months
	38		2,700	20,000	15,000	6,000		100	4,500	4,500	550		No information

colonies of lactobacilli were identified and counted with the aid of a binocular dissecting microscope and a Frost counting card for a background.

Table I shows that of the 38 children selected as caries-free the salivas of 16 were positive for lactobacilli at the time of the first culture. The significance of this finding in relation to the dental condition will be discussed later. However, as the study progressed, we found in the Maumee group of 17 subjects only 1 who had persistently large numbers of lactobacilli in the saliva. Two other sporadic positives were noted. On the whole the mouths of this group were almost free from lactobacilli and caries, although there were the exceptions of the 1 case with the lactobacilli and no caries and the extension of 1 lesion in a mouth with no lactobacilli. Unfortunately this extension was not found until 3 months after the last specimen from this individual was cultured, and it was possible that the lesion was formed in that period. It is interesting to compare these results with the numbers of lactobacilli and the dental condition at the end of the study of similarly selected caries-free Ann Arbor children of approximately the same ages. Despite the exceptions the data indicate a correlation between the presence and increasing numbers of lactobacilli and the caries present. There were 9 cases listed as "doubtful-negative" for

caries because the explorer caught at one point during the examination but no lesion was demonstrable. For summation purposes they were

TABLE II

Numbers of yeasts in specimens of saliva from selected caries-free children cultured irregularly over an eighteen month period

GROUP	NUMBER	NUMBER OF CULTURES											DENTAL CONDITION AT END OF STUDY
		1	2	3	4	5	6	7	8	9	10	11	
Maumee	1	0	0	0	0	0	0	0	0	0			Negative 6/1/38—8 months
	2	0	0	0	0	0	39,000	0	0				Went home
	3	135	0	0	0	0	6,000	0	0	600	25,000		Negative
	4	0	0	0	0	0	0	0	0	0	0		Negative
	5	0	0	0	0	0	0	0	0	0	0		Negative
	6	0	0	0	0	0	0	0	0	0	0		Negative
	7	1,500	2,400	1,150	750	900	3,000						Went home
	8	1,200	2,100	24,000	200	200	200	0	250	0	10		One small extension
	9	125	150	230	125	100	22	5	0	0	0		Doubtful—negative
	10	10	0	0	5	0	10	0	0	0	0		Negative
	11	0	0	0	0	0	0	0	0	0	0		Negative
	12	0	0	0	0	0	0	4	0	0	0		Negative
	13	0	0	0	0	0	0	36	0	0	0		Negative
	14	0	0	0	0	0	0	0	0	0	0		Negative
	15	0	0	0	0	0	0	0	0	0	0		Negative
	16	0	0	0	0	0	0	0	0	0			Negative—6 months 6/1/37
	17	0	0	0	0	0	0	0	0				Went home
Ann Arbor	18	0	0	0	0	0			0				No information
	19	0	0	0	0	0	0	0	0	0	0	0	4 cavities, 2 pits, 2 fillings
	20	0	0	0	0	0	0	0	0	0	0	0	3 cavities
	21	0		0	0	0	0			0	0	0	Doubtful -negative
	22	0	0	0		0	0	0	0	0	0	0	Negative
	23	4,950		0		150	0	0	0	150	85	0	14 cavities
	24	2,700		0	0		0	0	0	0	0		Caries? 6/1/37, definite caries 6/1/38
	25	0	0	0	0		0	0	0		0	0	Doubtful -negative
	26	0		0	0	0	0	0	0	0	0		Negative
	27	0		0	0	0	0	0	0	0	0	0	Doubtful -negative
	28	0		0	0	0	0			0	0	0	Doubtful -negative
	29		0	0		0	0	0	0	0	0	0	Doubtful -negative
	30		0	0	0	4	0	0	0	0	0	0	Negative
	31		0	0	20,000		20,000			0	0		2 small cavities
	32		0		5	0	0	0	0	0	0		Negative
	33		0	0	0	0	0	0	0	0	0		Doubtful -negative
	34		125	5	28	23	0	10	20	220	0		Small amount of caries
	35		0	0	0	0	0		0	0	0		Doubtful -negative
	36		45	0	0	450	1,200		100	75	0		Doubtful -negative
	37		0	0	0			0	0	0			Negative 6/1/37—8 months
	38		0	0	0	0		0	0	0	0		No information

considered negative, although the necessity for further study of these children is obvious. When these data were subjected to statistical

TABLE III

Numbers of streptococci in specimens of saliva from selected caries-free children cultured irregularly over an eighteen month period

GROUP	NUMBER	NUMBER OF CULTURES											DENTAL CONDITION AT END OF STUDY
		1	2	3	4	5	6	7	8	9	10	11	
Maumee	1	0	0	130	136	35	0	0	750	0			Negative 6/1/37—8 months
	2	0	0	900	4,500	0	0	0	0				Went home
	3	0	0	0	0	0	0	0	0	0	0		Negative
	4	0	0	0	0	0	11,000	0	0	0	0		Negative
	5	0	0	0	3,000	0	10,000	8,250	450	0	30,000		Negative
	6	0	0	1,350	45	180	50,000	0	0	0	0		Negative
	7	0	0	200	300	600	5,250						Went home
	8	0	0	0	0	0	0	0	0	0	25,000		One small extension
	9	0	0	0	2,700	450	3,000	0	0	70	0		Doubtful—negative
	10	0	0	150	150	0	0	0	0	0	0		Negative
	11	0	0	0	0	0	0	0	0	0	10,000		Negative
	12	0	0	150	0	30	9,000	0	250	0	50,000		Negative
	13	0	0	30,000	4,800	0	0	0	0	0	0		Negative
	14	0	0	75	0	225	0	0	0	0	0		Negative
	15	0	0	0	0	10,500	0	0	0	0	10,000		Negative
	16	0	0	4,000	0	225	0	0	0	0			Negative 6/1/37—8 months
Ann Arbor	17	0	0	200	125	0	0	0	750				Went home
	18	0	0	0	0	0			0				No information
	19	0	0	0	250	0	0	0	0	0	0	0	4 cavities, 2 pits, 2 fillings
	20	0	0	0	0	0	0	0	0	0	0	0	3 cavities
	21	0		0	0	0	0			600	0	0	Doubtful—negative
	22	0	0	0		0	0	0	0	0	0	0	Negative
	23	0		0		0	0	0	0	0	0		14 cavities
	24	0		1,800	0		0	0	0	0	0		Caries? 6/1/37, definite caries 6/1/38
	25	0	0	0	0		0	0	0		0	0	Doubtful—negative
	26	0		0	12,000	0	0	0	0	0	0		Negative
	27	0		0	0	3,000	0	0	0	0	0	0	Doubtful—negative
	28	0		0	38	0			0	0	0	0	Doubtful—negative
	29		0	0		0	1,500	20	3,000	0	0	0	Doubtful—negative
	30		0	1,350	0	0	0	0	4,500	0	0	0	Negative
	31		0	0	0		0			0	0		2 small cavities
	32		0		0	0	0	0	6,000	0	0		Negative
	33		0	0	0	0	0	0	0	0	0		Doubtful—negative
	34		0	120	60,000	0	0	0	60,000	0	0		Small amount caries
	35		0	0	60,000	0	0		0	0	0		Doubtful—negative
	36		0	0	0	10	0		0	150	0		Doubtful—negative
	37		0	0	450			0	12,000	0			Doubtful—negative
	38		0	0	0	0		0	7,500	0	0		No information

analysis[3] it was found that the occurrence of lactobacilli in respect to cavity formation was significant, but on the basis of correlation coeffi-

[3] The author is indebted to Dr. Paul Dwyer, Assistant Professor of Mathematics, for assistance in the statistical analysis.

TABLE IV

Numbers of staphylococci in specimens of saliva from selected caries-free children cultured irregularly over an eighteen month period

GROUP	NUMBER	NUMBER OF CULTURES											DENTAL CONDITION AT END OF STUDY
		1	2	3	4	5	6	7	8	9	10	11	
Maumee	1	0	12	128	125	5	0	600	600	0			Negative 6/1/37—8 months
	2	280	0	3,750	18,000	2,500	280	24,000	8,000				Went home
	3	0	0	5,300	0	450	750	1,200	1,200	600	160		Negative
	4	0	0	8,400	3,000	750	4,850	0	3,000	0	1,800		Negative
	5	0	0	0	0	0	0	0	0	0	0		Negative
	6	0	0	0	5	35	35	0	750	150	900		Negative
	7	50	25	1,000	0	300	600						Went home
	8	55	20	15,000	200	80	0	750	170	1,800	250		One small extension
	9	0	0	67	80	0	18	750	170	1,800	250		Doubtful—negative
	10	30	25	40	10	125	10	62	25	0	20		Negative
	11	0	0	150	60	65	0	2,250	38	0	450		Negative
	12	0	20	16	4	25	60	3,100	10	125	0		Negative
	13	0	0	30,000	6,600	20,000	3,750	6,000	1,500	0	600		Negative
	14	0	75	5	125	0	0	30	0	10,800	0		Negative
	15	0	0	65	12	1,500	3,600	9,600	500	0	350		Negative
	16	0	0	3,500	125	225	36,000	3,000	5,000	600			Negative 6/1/38—8 months
	17	0	0	2,000	300	1,000	900	60,000	12,000				Went home
Ann Arbor	18	0	0	0	0	3,000			150				No information
	19	0	0	0	24,000	1,800	28	0	900	0	0	0	4 cavities, 2 pits, 2 fillings
	20	0	5	2,700	250	0	0	200	900	0	0	0	3 cavities
	21	0		130	150	180	180			1,050	60	0	Doubtful negative
	22	0	0	300		0	0		1,200	40	0	0	Negative
	23	0		0		2,500	0	0	0	0	0	0	14 cavities
	24	0		3,600	0	0	0	0	6,000	0	0	0	Caries? 6/1/37, definite caries 6/1/38
	25	600	0	0	1,050		550	2,400	15,000		0	0	Doubtful—negative
	26	0		12	10,200	10	375	4,500	1,200	0	0		Negative
	27	0		2,175	750	1,800	0	12,000	4,500	0	0	0	Doubtful—negative
	28	0		4,000	15	0	65			0	450	20	Doubtful—negative
	29		0	318		200	120	15,000	0	0	0	0	Doubtful—negative
	30		0	375	25	85	0	1,500	150	0	0	0	Negative
	31		0	33,000	0		0			0	0		2 small cavities
	32		4		225	250	0	4,500	0	0	0		Negative
	33		0	0	4	0	0	0	0	10	0		Doubtful—negative
	34		0	0	2,375	0	1,425	100	35	0	0		Small amount of caries
	35		0	1,200	925	65	600		600	0	0		Doubtful—negative
	36		0	450	365	20	450		1,500	50	0		Doubtful—negative
	37		0	15	178			2,400	600	75			Negative 6/1/37—8 months
	38		0	0	0	0		250	0	600	0		No information

cients it was possible to predict caries for the group only rather than the individual.

There remain for consideration, however, the other acidogenic

organisms in the mouth. In view of the controversy existing over the role of these forms in dental caries and the fact that there are no extensive and parallel figures on their occurrence and relative numbers, other than lactobacilli, in the saliva as determined by a selective medium over a given period, it seemed highly necessary to tabulate their frequency of appearance on the acid tomato-peptone agar. That the medium was highly selective may be noted by the many culturally negative plates and the very sparse growth of some of the millions of streptococci and other organisms in the saliva. These aciduric forms included the yeasts, streptococci, and staphylococci with the sarcines; their frequency is recorded in Tables II, III, and IV, For the reason of space a group of miscellaneous types is not given.

The data presented in these tables show that the incidence and numbers of streptococci and staphylococci as determined by growth on acid tomato-peptone agar had little if any relation to the caries observed. However, the yeasts warrant some consideration since these forms were frequently present in the salivas of the children with caries. Although the Maumee child with the 1 cavity had apparently significant yeast counts and no lactobacilli, the relation becomes doubtful since the yeasts decreased in number toward the latter part of the study and the lesion was not observed until 3 months after the last specimen was cultured. Similarly, the yeast cells in the salivas of the Ann Arbor children with caries appeared sporadically and seldom in appreciable numbers. These facts, combined with the observation (data in press) that yeasts apparently produce little additional acid in glucose infusion agar at pH 5.0 as measured by changes in the indicator, brom-cresol-green, make it appear that the role of yeasts in dental caries is supplementary to the lactobacilli. These conclusions are supported by statistical analysis with the exception that by the use of correlation coefficients the number of staphylococci in respect to the number of cavities approaches the minimum significant figure (0.3).

DISCUSSION

To interpret any information obtained by cultural studies it must be remembered that it is not possible to tell whether caries is active or arrested by merely looking in the mouth or examining extracted teeth.

Only by careful dental examination followed by others at regular intervals over a period of a year when any extension of previous lesions or appearance of new decay can be noted may activity be established. The lack of adequate clinical information eliminates from serious consideration many of the reported bacteriological studies of dental caries. This omission is especially noticeable in the recent papers of Anderson and Rettger (16) and Tunnicliff and Hammond (22). The former found lactobacilli in only 12 per cent of 65 cases selected apparently without sufficient dental information; the latter in their few examinations depended almost entirely on extracted teeth, and assumed caries activity by the presence of cavities. However, where carefully controlled comparisons of cultural and clinical findings have been made as in the present paper, the Michigan group (4–1), Jay (24), Rosebury and Greenberg (25), Enright, Friesell and Trescher (26), and others, a correlation between the numbers of lactobacilli and the caries observed in the subjects may be demonstrated. Yet in any group, even as in the present selected caries-free children, cultures of the salivas will show certain individuals who are either consistently positive or negative for lactobacilli, some who are becoming free from lactobacilli, and others in whose mouths lactobacilli are beginning to appear. On a clinical basis these results would be interpreted as definite caries or caries expectancy for the continued or intermittent positives, and freedom from decay for the negatives or those subjects entering a lactobacillus free period. Using the numbers of lactobacilli as an index, Jay (33) feels able with reasonable accuracy to forecast the expectancy of new or recurrent caries.

In this study the Maumee group of 17 children remained on the whole free from lactobacilli and caries. One child with no lactobacilli in the specimens of saliva cultured was found to have a slight extension of a previous lesion at the dental examination 3 months after the last saliva sample was taken. The possibility of extension in that time interval makes this exception of doubtful significance. One other child had numerous lactobacilli at every examination but no caries. The low incidence of caries in this home in spite of a caloric and vitamin deficient diet is remarkable. The seemingly important factor in their diet is the nearly complete absence of refined sugar. As long as the children are in the home, they remain usually free from

lactobacilli and caries is practically non-existent. That these individuals are not naturally resistant or immune to caries may be discerned from the candy feeding experiments recorded by Jay, Hadley, Bunting and Koehne (34), and by the fact that many of these children had caries previous to entering the home.

Of the 21 Ann Arbor children selected as caries-free, dental information was available for only 18 at the middle and end of the period. Six children had definite caries associated with significant lactobacillus counts. Three had no caries but their salivas usually contained appreciable numbers of lactobacilli. Five were negative for lactobacilli and were free from caries activity. The remaining 4 showed sporadically positive salivas but were caries-free. The concluding dental and bacteriological examinations showed, therefore, a fair amount of caries and a tremendous increase in the occurrence of lactobacilli in the saliva. For those individuals with no caries and persistent lactobacilli in the saliva, the necessity for further observation is obvious.

Among the other acidogenic or aciduric forms isolated or observed, the staphylococci and sarcines were apparently eliminated from consideration because they were about equally distributed in carious and non-carious mouths. The streptococci did not seem to have any relation to the caries observed, since there were more streptococci from the salivas of the caries-free Maumee children than from the Ann Arbor group which was not caries-free. Although Anderson and Rettger (16) showed that streptococci decalcified the enamel of teeth *in vitro* and felt that streptococci should not be disregarded in the caries problem, it can hardly be said that the conditions of the test tube are duplicated in the mouth. For a detailed review of the problems of *in vitro* decalcification in relation to the disease the admirable paper of Enright, Friesell and Trescher (26) should be consulted. These workers arrived at the conclusion that lactobacilli were the most important bacteria involved in dental caries, but they are quoted incompletely and to the contrary by Anderson and Rettger. Furthermore, our experience, as well as others, has been that when cultures of saliva or teeth are made in glucose broth or glucose brain broth the streptococci always present will grow very rapidly but will shortly decrease in numbers under the acid produced and will be

overgrown by the lactobacilli if present. This situation unquestionably explains Tunnicliff and Hammond's recovery of bacilli which they assumed to be rough variants of streptococci, from glucose broth cultures of dentin and pulps. The impurity of their cultures is all the more emphasized by the isolation of streptococci from the bacilli after several rapid transfers but inability to do the same with pure line strains of *L. acidophilus*. Their conclusion that streptococci were the invaders in dental caries no matter what the predisposing factors was weakened also by the fact that their cultures were limited to 6 pulps, 6 dentins, and 2 specimens of saliva.

The yeasts provoked interest because they were the only organism present in significant numbers in the mouth of the Maumee child with an extension, and they were also found frequently in 3 of the 6 Ann Arbor children with caries. These observations bring up the work of Fosdick and Hansen (42) who found that the rate of calcium disintegration in saliva and carbohydrate mixtures increased 100 per cent by the addition of yeasts to the lactobacillus cultures. Thus, it is probable, that while yeasts do not under ordinary circumstances cause tooth decay, they hasten the process. In such a way the problems of synergism are presented by the legion and variety of bacteria in the mouth and the combined action of their end products about which we have little or no information.

SUMMARY

The occurrence of lactobacilli in the salivas of selected caries-free children was observed over an 18 month period. In the salivas of 17 children from an institution in Maumee, Ohio, only 11 of 159 specimens were positive for lactobacilli, whereas of 178 specimens of saliva from 21 Ann Arbor public school children 85 were positive for lactobacilli. Similarly, the teeth at the end of the study showed only 1 Maumee subject to have a small extension, the significance of which was questioned, while 6 Ann Arbor children were definitely subject to caries. Although as individuals there were several apparent exceptions, as the one Maumee and several Ann Arbor subjects who had lactobacilli more or less consistently present in their salivas but had no caries, it must be remembered that these children were carefully selected as caries-free in the beginning and did not represent a cross-

section study. It is obvious that further individual studies should be made. Nevertheless, as groups divided on the basis of lactobacillus population, those children who had no lactobacilli in their salivas at the time of collection remained free from caries, and those who had significantly appreciable numbers of lactobacilli in the saliva showed varying amounts of decay. Other aciduric organisms were isolated, but of the yeasts, streptococci, and staphylococci only the yeasts seemed to have any relation to the caries observed and this role was apparently secondary to that played by the lactobacilli.

REFERENCES TO LITERATURE

1. Miller, W. D., *Die Mikroorganismen der Mundhöhle.* (Leipzig, George Thieme: 1889.)
2. Kligler, I., *J. Allied D. Assoc.*, **10**: 141, 282, 445, 1915.
3. Rodriguez, F. E., *Military D. J.*, **5**: 199, 1922.
4. Bunting, R. W., and Palmerlee, F., *J. A. D. A.*, **12**: 381, 1925.
5. Bunting, R. W., Nickerson, G., and Hard, D. G., *D. Cosmos*, **68**: 931, 1926.
6. Bunting, R. W., Nickerson, G., Hard, D. G., and Crowley, M., *D. Cosmos*, **70**: 1, 1928.
7. Bunting, R. W., Hadley, F. P., Jay, P., and Hard, D. G., *Am. J. Dis. Child.*, **40**: 536, 1930.
8. Bunting, R. W., Nickerson, G., Hard, D. G., and Keller, M., *D. Cosmos*, **70**: 1002, 1928.
9. Hadley, F. P., Bunting, R. W., and Delves, E. A., *J. A. D. A.*, **17**: 2041, 1930.
10. Hadley, F. P., and Bunting, R. W., *J. A. D. A.*, **19**: 28, 1932.
11. Koehne, M., Bunting, R. W., and Morrell, E., *Am. J. Dis. Child.*, **48**: 6, 1934.
12. Morishita, T., *J. Bact.*, **18**: 181, 1929.
13. Rettger, L. F., and Horton, G. D., *Centbl. f. Bakt.*, **73**: 362, 1914.
14. Hunt, G. A., and Rettger, L. F., *J. Bact.*, **20**: 61, 1930.
15. Weinstein, L., Anderson, T. G., and Rettger, L. F., *J. D. Res.*, **13**: 323, 1933.
16. Anderson, T. G., and Rettger, L. F., *J. D. Res.*, **16**: 489, 1937.
17. Goadby, K., *Brit. Med. J.*, **2**: 769, 1910.
18. Clark, J. K., *Brit. J. Exp. Path.*, **5**: 141, 1924.
19. Baumgartner, E., *Wien. kl. Woch.*, **26**: 178, 1913.
20. Kantorowicz, S., *Deut. Zahn.*, **21**: 3, 1911.
21. Hartzell, T. B., and Henrici, A. T., *J. N. D. Assoc.*, **4**: 477, 1917.
22. Tunnicliff, R., and Hammond, C., *J. A. D. A.*, **25**: 1047, 1938.
23. McIntosh, J., James, W., and Lazarus-Barlow, P., *J. Brit. D. Assoc.*, **43**: 728, 1922.
24. Jay, P., *J. A. D. A.*, **16**: 230, 1929.
25. Rosebury, T., and Greenberg, S., *J. D. Res.*, **12**: 430, 1932.
26. Enright, J. J., Friesell, H. E., and Trescher, M. O., *J. D. Res.*, **12**: 759, 1932.
27. Tucker, W. H., *J. Inf. Dis.*, **51**: 444, 1932.
28. Johnston, M. M., Kaake, M. J., and Agnew, M. C., *J. A. D. A.*, **20**: 1777, 1933.

29. Nizzle, A., *Handbuch der pathogenen Mikroorganismen.* Kolle-Wassermann, **6**: 391, 1929.
30. Cruickshank, J., and Cruickshank, R., *System of Bact.*, **8**: 334, 1931.
31. Johnston, M. M., Williams, C. H. M., Anderson, P. G., Drake, T. G. H., Tisdall, F. F., and Kaake, M. J., *J. A. D. A.*, **23**: 1493, 1936.
32. Hadley, F. P., *J. D. Res.*, **13**: 415, 1933.
33. Jay, P., Bacteriology and Immunology of Dental Caries. *Dental Science and Dental Art.*, Gordon, S. editor., (Phila. Lea & Febiger: 1938).
34. Jay, P., Hadley, F. P., Bunting, R. W., and Koehne, M., *J. A. D. A.*, **23**: 846, 1936.
35. Fosdick, L. S., and Hansen, H. L., *J. A. D. A.*, **23**: 401, 1936.

ACID-PRODUCTION AS A BASIS FOR CLASSIFYING LACTOBACILLI FROM CARIOUS TEETH

H. R. SULLIVAN,[1] J. L. STILL,[2] AND N. E. GOLDSWORTHY

From the laboratories of the Department of Bacteriology, University of Sydney, Australia

INTRODUCTION

The majority of the more recent investigations of bacteria associated with carious lesions have emphasised the importance of lactobacilli. Probably the best known work is that of Bunting and the Michigan group (1936), who claim that there is a direct relationship between the numbers of lactobacilli present in the saliva and the amount of active caries of the teeth. In their classification of the oral lactobacilli the Michigan group described three types, all of which were included in their total counts, although these types had quite different physiological activities.

If we assume that enamel is a non-vital and practically wholly inorganic substance, it is obvious that the only organisms likely to produce lesions in it are those possessing strongly acidogenic properties. Enright, Friesell and Trescher (1932) realised this and stated that only one type of oral lactobacillus had the properties necessary for the production of such lesions. We decided therefore to investigate the various types of oral lactobacilli, giving particular attention to their ability to produce acid from fermentable carbohydrates.

Although this study is concerned solely with lactobacilli, we do not wish to imply that in the present state of knowledge these organisms can be regarded as the only bacterial factor in the initiation of caries.

As this genus has somewhat indefinite boundaries, we decided to regard as a lactobacillus any gram-positive organism of a bacillary form that was capable of growth on the modified tomato medium

[1] This work was carried out during the tenure of the Carnegie Dental Research Scholarship and later under a grant from the National Health and Medical Research Council of Australia.

[2] Department of Physiology.

described below. A similar basis of classification was used by Anderson and Rettger (1937).

MATERIALS AND METHODS

1. *Isolation of the organisms. The material:* All strains (more than 100) of *L. acidophilus* used were obtained from freshly-extracted teeth (to which no antiseptic had been applied) in the following manner:—the soft superficial layer over the carious area was removed, and part of the underlying carious material was taken by means of a sterile excavator. This was transferred to a sterile test tube and suspended in a small quantity of sterile distilled water (approximately 0.5 mil.).

The media: In most cases the suspension was then plated on to Kulp's (1927) tomato peptone agar as modified by Kulp and White (1932) with a pH value of 5.0. This medium was largely selective for *L. acidophilus*; the only other types of organisms observed were aciduric cocci and yeasts. Occasionally the suspension was placed into tubes of broth with a pH value of 3.5 and after 3 days' incubation the growth was plated onto glucose agar. In both cases the colonies were examined with a dissecting microscope when the cultures were 3 days old, and a full description recorded. Single colonies were then picked and transferred to 0.5 per cent glucose agar slopes, which were incubated for 2 days. Further cultures were made from these slopes and tested for purity (by replating and by examining stained films). The morphology was carefully observed.

Anaerobic cultivation: As freshly-isolated cultures of *L. acidophilus* are known to be preferably microaerophilic or even anaerobic, most of the work (both isolation and the subsequent tests) was done under anaerobic conditions, and all tests were begun within a fortnight of isolation, in order to avoid possible effects of "acclimatisation" due to long residence in artificial culture media.

2. *Study of the organisms.* The organisms were investigated primarily to determine their acid-producing properties. In order to link up our work with that of others we studied also the colonial features, the cellular morphology, and the fermentations on a wide range of substances. The behaviour of the organisms on bile media was also observed.

(a) *Tests for acid-production:* Three such tests were applied, generally in the order indicated.

(i) *Appearance of cultures on serum glucose agar plates:* Pure cultures were inoculated on to nutrient agar containing 10 per cent normal horse serum and 1 per cent glucose. After incubation (anaerobically) for 3 days the plates were inspected for precipitation of serum protein. When precipitation occurred, the medium appeared opaque, especially at the site of heavy growth.

(ii) *Ability to lower the pH value of a medium containing fermentable carbohydrate:* The organisms were tested in the following way. Two drops of a broth suspension (made from a 48-hour agar slope culture) were inoculated into tubes of nutrient broth containing added carbohydrate as follows: (α) 1 per cent glucose, (β) 1 per cent maltose, (γ) 1 per cent saccharose, (δ) 1 per cent dextrin. After incubation at 37°C. for 48 hours the change in the reaction of the media was determined colourimetrically by means of a B.D.H. "Capillator" set. About a dozen strains were tested under both aerobic and anaerobic conditions in order to be certain that the variation in oxygen tension did not affect the result. As no difference was observed, subsequent experiments of this type were incubated only anaerobically.

(iii) *Determination of the amount of acid produced from fermentable carbohydrate:* Erlenmeyer flasks containing 200 mils. of ½ meat extract broth[2] with 1 per cent added glucose were inoculated with 1 mil. of peptone water suspensions of individual strains (approximately 1000 millions per mil.). The flasks were then incubated aerobically at 37°C. Control flasks (no added glucose) were always incubated at the same time in order to be sure that the acid produced by the organisms was derived from the added carbohydrate. At the end of each 24-hours 10 mils. were removed and the amount of N/20 NaOH required to bring the pH to 6.5 (i.e. the original value) was determined with bromcresol purple as indicator. A tube containing 10 mils. of the original medium with indicator added was kept and used as a colour control until the end of the experiment. The amount of sterile normal sodium hydroxide necessary to restore the pH of the culture to 6.5 was calculated and added. The experiment was ended when no more acid was formed and the cultures were then tested for purity by the examination of stained films and of subcultures on serum glucose agar plates.

(b) *Colonial features:* Colonies were studied principally on the tomato medium, but difficulty was experienced in assigning many colonies to the groups described by other workers.

(c) *Morphology:* This was studied in films prepared from 72-hour cultures in glucose broth and on tomato peptone agar. Films were stained by Gram's method, corresponding in the main with that described by Wadsworth (1927).

(d) *Fermentation reactions:* The peptone water base commonly used for fermentation tests was found to be unsuitable for these organisms because of the poor growth (often imperceptible) in this medium. We therefore used a medium prepared as follows:—100 gm. of minced ox heart, 5 gm. of sodium chloride, and 10 gm. of peptone were added to 1000 mils. of distilled water and placed in a steamer at 68°C. for 10 minutes, after which the temperature was raised to 100°C. and held there for ½ hour. After filtering through Postlip paper and adjusting the pH to 7.0 the medium was again steamed for ½ hour and filtered. Andrade's indicator was added and the medium tubed and autoclaved at 10 pounds' pressure for 10 minutes. This medium is referred to as "½ meat extract broth." It did not reduce Fehling's reagent, whereas the ordinary nutrient broth showed some reduction. In this medium we were able to get a moderate growth of the organism and at the same time to avoid false results due to the carbohydrate content of ordinary nutrient broth. Tests were carried out on glucose, maltose, lactose, saccharose, mannitol, dulcitol, salicin, raffinose, inulin, dextrin and starch. A large inoculum (3 drops of a suspension of approximately 1000 million organisms per mil.) was used and the cultures were incubated anaerobically, readings being taken on the fourth and seventh days. The control tubes were similar in every way except that no carbohydrate was added.

(e) *Growth on media containing sodium taurocholate:* To nutrient agar were added 0.25 per cent glucose and sodium taurocholate in a concentration of 5 per cent to 7.5 per cent. A moderately heavy inoculum was sown on to plates of this bile medium and after 3 days' incubation the cultures were observed for inhibition of growth.

RESULTS

(a) *Tests for acid-production.* (i) *Appearance of cultures on serum glucose agar plates:* Strains either caused an obvious opacity of the

[2] See under-(d) Fermentation reactions.

erstwhile clear medium or did not affect it visibly. Occasionally a strain caused only slight clouding of the medium. The change in the medium was always most noticeable where the growth was heaviest.

(ii) *Ability to lower the pH value of a medium containing fermentable carbohydrate:* Some strains increased the acidity of the medium containing 1 per cent glucose from pH 7 to below 5. In most of these cultures the value attained was below 4. Other strains, however, failed to bring the pH value below 5.4.

TABLE I

The pH value of cultures containing various carbohydrates, after 48 hours at 37°C. (initial pH value of the media—7.0)

STRAIN*	1 GLUCOSE	2 MALTOSE	3 SUCROSE	4 DEXTRIN	5 CONTROL
106	4.1	5.2	4.6	6.3	6.6
108	4.0	6.0	5.6	6.4	6.6
110	3.9	4.2	5.1	6.0	6.4
114	4.1	4.0	4.2	5.2	6.5
140	3.9	6.0	4.0	6.3	6.4
148	3.9	6.1	4.0	6.6	6.3
122	5.4	6.2	5.2	6.6	6.5
123	6.2	4.6	5.2	6.6	6.5
129	5.5	5.9	5.8	6.4	6.5
134	6.2	5.2	6.8	6.4	6.6
137	6.4	6.4	6.4	6.5	6.4
144	6.2	6.2	6.4	6.3	6.4

* For the first 6 strains the pH value of the glucose medium is well below 5.0, whereas for the remaining 6, it is above 5.0. The value for glucose (column 1) is not indicative of the activity of any given strain towards other carbohydrates (columns 2, 3, and 4).

There was no complete parallelism between the activity of any individual strain in media containing glucose and in media containing other carbohydrates. Of these other carbohydrates, maltose was attacked most often and to a greater degree, whilst dextrin was very rarely affected. Table I shows figures for several typical strains.

(iii) *Determination of the amount of acid produced from fermentable carbohydrate:* Tables IIA and IIB and *fig. 1* show the results of this type of experiment. It is clear from the values set out, that there is considerable variation in the activity of various strains towards glucose. The variation is seen to be twofold, involving both the rate of production of acid and the total amount produced (*fig. 1*).

TABLE IIA

The total amount of N/1 NaOH needed to maintain the pH value of glucose broth cultures (200 ml.) at 6.5

EXPERIMENT	STRAIN	N/1 NaOH ADDED	N/1 NaOH ADDED TO CONTROL—NO GLUCOSE	PERIOD OF GREATEST ACTIVITY OF ORGANISMS	TOTAL PERIOD OF ACTIVITY OF ORGANISMS
I	130	15.8 ml.		3 days	5 days
	132	15.81 "	0.3 ml.	4 "	5 "
	137	7.82 "		4 "	7 "
II	115	16.71 "	0.1 "	3 "	4 "
	112	7.74 "		7 "	7 "
III	85	17.87 "	0.3 "	3 "	3 "
	92	6.7 "		4 "	4 "
IV	10a	17.03 "	0.3 "	4 "	7* (4) days
	10c	3.08 "		3 "	3 "
V	148	17.6 "	0.55 "	2 "	3 "
	144	5.95 "		8 "	8 "

* See note under Table IIB.

TABLE IIB

Summary of results shown in Table IIA

	STRAINS PRODUCING MUCH ACID	STRAINS PRODUCING LITTLE ACID
Average amount of N/1 NaOH added	16.81 mls.	6.26 mls.
Average amount of N/1 NaOH added to control flask (no added glucose)	0.31 ml.	—
Average total period of activity	4.5 days (4.0 ")*	5.8 days
Average period of greatest activity	3.2 days	5.2 days

* For strain 10a the figure 7 in the last column of Table IIA could be replaced for all practical purposes by the figure 4, since the change observed during the last 3 days was negligible. If we accept the figure 4, then in Table IIB the average period of activity for the group concerned would be reduced from 4.5 to 4.0 days.

(b) *Colonial features:* We found no difficulty in recognising the white, shiny, domed colony, usually referred to as the Y type (Rettger and Horton 1914). There were however numerous other colonies

(some resembling the rough grey type described by Bunting) which did not conform to any of the previously established types.

(c) *Morphology:* This showed great variation and was in no way correlated with the colonial forms or the physiological reactions of the strains. This result is in agreement with the opinions of Enright, Friesell and Trescher, who deny that morphology has any useful application in grouping these organisms.

(d) *Fermentation reactions:* In the following paragraphs the arrangement of the carbohydrates in groups is purely one of convenience.

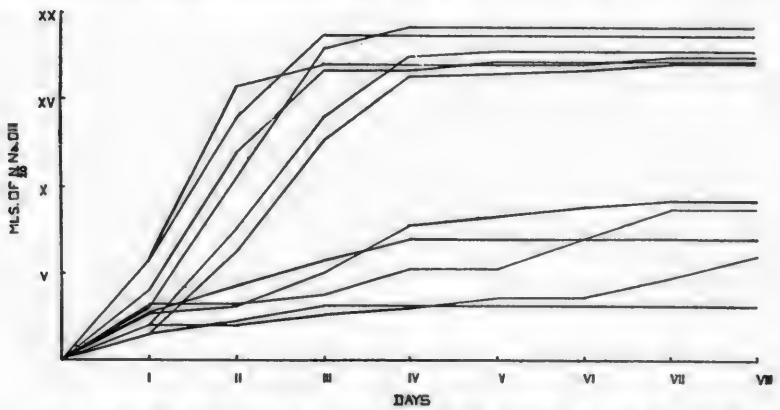


FIG. 1. Showing that strains fell into 2 clearly defined groups. In one acid production was rapid and high, in other it was much slower and much less. Curves are based on amounts of N/20 NaOH required to neutralize 10 ml. samples of cultures. Height of curve at any particular day represents the sum of all amounts needed, up to and including that day.

(i) *Glucose*, *maltose*, *lactose* and *saccharose* were as a rule readily fermented. Saccharose showed the greatest number of exceptions:—only 89 of 103 strains gave a positive result. Lactose had 12 exceptions, maltose 10 and glucose only 2. That there were these exceptions is possibly to be explained on the ground that some strains seem to be very exacting in their growth requirements.

(ii) *Dulcitol*, *inulin*, *starch* and *dextrin* gave completely negative results, except that 9 of 93 strains tested attacked the dextrin slightly. Therefore these substances were omitted from the series of test carbohydrates.

(iii) *Mannitol* was fermented by only 12 of 103 strains when tests were incubated anaerobically. Since this was in direct contrast to the results obtained by other workers, we tested 72 strains under aerobic conditions. Fifty-five of the 72 were known to be capable and the other 17 to be incapable of precipitating serum protein in serum glucose agar medium and of lowering the pH value of glucose broth below

TABLE III

A comparison of the physiological activities of the groups

	TEST	S.A. GROUP		W.A. GROUP	
i.	Production of opacity on serum glucose agar	+		−	
ii.	pH of glucose broth culture after 48 hours' incubation (original pH of broth = 7.0)	Less than 5.0 (generally less than 4.5)		More than 5.0 (generally more than 5.5)	
iii.	Amount of N/1 NaOH required to neutralize acid produced in 1% glucose broth (compare *fig. 1*)*	More than 16 mls.		Less than 8 mls.	
		+	−	+	−
iv.	Fermentation of				
	(a) Salicin	93.7	6.3	41.2	58.8
	(b) Raffinose	23.4	76.6	82.4	17.6
	(c) Mannitol	81.8	18.2	23.5	76.5
v.	Growth on medium containing sodium taurocholate (5–7.5 per cent)**	96.6 per cent		18.2 per cent	

* Only 6 S.A. strains and 5 W.A. strains were tested in this way.
** Only 29 S.A. strains and 11 W.A. strains were tested in this way.

5. Of the 55 active strains 45 fermented the mannitol; whereas only 4 of the 17 relatively inactive strains attacked this carbohydrate. This appeared to be the only test where a variation in oxygen tension affected the result.

(iv) *Raffinose* and *salicin* gave results whose relation to those of other tests is shown in Table III.

(e) *Growth on bile salt glucose agar:* The strains either grew moderately well or were completely inhibited.

(f) *Summary of results:* One hundred and three strains were tested for their acidogenic capacity. Each strain fell into one of two clearly defined groups: either it was strongly acidogenic (the "S.A." group) or it was weakly acidogenic (the "W.A." group). Of the 103 strains 67 were strongly acidogenic and 36 were weakly acidogenic. The correlation of the various tests for acid production with the other physiological properties of oral lactobacilli is shown in Table III. Since colonial form and cellular morphology were so variable and unreliable no attempt has been made to include these features in the table.

DISCUSSION

Although lactobacilli have been recognised as a distinct group for over fifty years, it is only during the last fifteen years or so that they have been seriously considered as a possible cause of dental caries. Most of the older papers dealt with strains from sources other than the oral cavity. Among the earlier attempts to establish an aetiological relationship between the lactobacillus and dental caries may be mentioned those of Howe and Hatch (1917) and McIntosh, James & Lazarus-Barlow (1922; 1924). It was only natural that the question of the identity of lactobacilli from various sources should be studied and that such classification as had been accepted for non-oral strains should be considered in relation to strains isolated from carious teeth.

Previous attempts to classify lactobacilli. In 1910 White and Avery described, on the basis of acid production, 2 types, A and B, of lactobacilli from various preparations of fermented milk. A few years later, Rettger and Horton (1914) likewise described 2 types of lactobacilli as a result of their study of the intestinal flora of rats. These types they distinguished as X and Y.

More recently Hadley, Bunting and Delves (1930) described 3 groups of aciduric organisms isolated from carious lesions. They suggested that their "smooth" type might be identical with Rettger and Horton's type Y, and their "rough" type, with type X.

About the same time Enright, Friesell & Trescher (1932) also studied oral lactobacilli along with a few strains from other sources

and came to the conclusion that most oral strains could be assigned to one of 2 main groups comparable to Rettger and Horton's X and Y types. What they termed their "commonly found dental strain" was in their opinion identical with the variety most commonly met by previous workers, e.g., Hadley, Bunting and Delves (1930), Rodriguez (1922) and Morishita (1929), and also with the Y type. Similarly their other main group appears to correspond to the X type.

A more comprehensive examination of lactobacilli generally was undertaken by Curran, Rogers and Whittier (1933). They concluded that only a broad grouping is possible and recognised (2) groups, A and B, in both of which dental strains were fairly evenly represented.

Ulicny (1936) disagreed with previous workers and considers dental strains uniformly more active physiologically than intestinal strains. Anderson and Rettger (1937) believed that oral strains are of one type only and likewise support the view that these belong to type Y and are therefore quite distinct from the intestinal strains which they assign to type X.

After reviewing the more accessible papers, these three points seem to emerge:—1. That classification of strains from whatsoever source or site has presented great difficulties which have not been lessened by the different nomenclatures and criteria used by various authors. 2. That there are two diametrically opposed schools of thought on the identity of the varieties of lactobacillus occurring in different sites, for example, the intestine and the oral cavity. 3. That there is fairly general agreement that among lactobacilli from all sources there are two main groups, the boundaries of which are somewhat ill defined.

Acid production as a basis for classification of oral strains. From a consideration of the work of Bunting and his colleagues and of Enright and his co-workers and of our own results we agree with Topley & Wilson (1936) that the *surface* colony form would appear to be too variable for use in classification. It is generally recognised that colony form is notoriously susceptible to minute variations of physical and chemical factors in the medium. We therefore favour the use of physiological criteria more especially because a classification based on biochemical activity appeared to us essential for the investigation of dental caries from a chemico-parasitic point of view.

In view of differences in the strains observed by earlier workers we have felt with Enright, Friesell and Trescher that different types of lactobacilli might well have different potentialities as decalcifiers of enamel. We therefore set out to discover any differences in acidogenic powers of the strains isolated from carious teeth, and to correlate, if possible, the results obtained by other workers with our own. As the latter show plainly, there are 2 main groups of oral strains (a) the strong acid producers, (b) the weak acid producers. These we have designated as S.A. and W.A. respectively.

Our evidence is based on three tests:—1. Alteration of appearance of serum glucose agar. 2. The change of pH in glucose broth. 3. Production of acid over a long period in glucose broth, daily restored to the original pH.

Of these tests, one (the reduction of pH in broth) has been used by many workers. Another test, precipitation of serum proteins, is described (in modified form) by Anderson and Rettger (1937) who however appear to lay emphasis on the colour change rather than on the protein precipitate (their medium contained brom-cresol-purple as well as serum). As a rule the distinction between the 2 types of organism was clear cut, but a small proportion of strains gave only very slight clouding of the medium. These strains generally lowered the pH of glucose broth to a level between 5.0 and 5.5.

The third test, viz. titration of the total quantity of free acid produced in glucose broth, was perhaps the most convincing of all. It bears some resemblance to the technique used by Avery and White (1910), but would seem superior because of the daily restoration of the reaction to a point near the optimum for the growth and function of the organisms. Nonetheless, there is a striking similarity between their curves and ours. Longworth and McInnes (1935) described an elaborate method for maintaining a constant pH value in a culture of lactobacillus over several days. Our own technique similarly aimed at preventing too great an accumulation of hydrogen ions but was obviously much less refined.

A study of the curves (*fig. 1*) and of Table IIB shows that there was (1) a distinct difference in the total amount of acid produced by the 2 types, and (2) a difference in the rate at which the organisms attacked the carbohydrate. Admittedly no attempt was made to correlate these figures with bacterial population of the flasks, so that

the average ability of the organisms to attack carbohydrate might well have been the same, although our other observations make this appear unlikely. One must consider here also the possibility that the organism which grows most rapidly and luxuriantly *in vitro* will flourish and be more formidable in the mouth. Rosebury (1932) has drawn attention to the correlation between the amount of growth and the final pH of the culture. It might be argued, and perhaps rightly so, that the S.A. strains produce more acid because they grow more vigorously in the particular medium used. This however is merely another characteristic difference between the S.A. and W.A. types, but because it is far easier to measure the hydrogen ion concentration than it is to count bacteria, we have not investigated this matter further. The subdivision made on the basis of the above tests will be seen to hold for the fermentation of various carbohydrates, but only in the broad way referred to by Curran, Rogers and Whittier. In other words, we met an appreciable proportion of strains which gave results suggestive of an overlapping of the groups.

Similarly the results with bile media showed a substantial difference between the S.A. and W.A. types: the S.A. type grew and the W.A. type did not, but as with the other tests, there were a few exceptions. Thus of 29 S.A. strains, 28 grew and one did not; of 11 W.A. strains only 2 grew, and 9 did not (Table III).

That such a classification as we have proposed into S.A. and W.A. groups, appears to bear a significant relationship to the problem of decalcification of enamel has been clearly indicated by experiments described in a separate paper. Sullivan prepared artificial plaques impregnated with S.A. and W.A. types respectively. Destruction of enamel occurred only if the plaque contained the S.A. type.

It is therefore obvious that a more accurate determination of the type of lactobacillus is necessary in any attempt to correlate lactobacilli with dental caries. The mere enumeration of lactobacilli without reference to their acidogenic potentialities is unlikely to be of much value unless the technique is such as to exclude the W.A. type without prejudicing the growth of the S.A. type. For this reason a method based on the principle underlying that used by Anderson and Rettger (1937) i.e. to permit the naked-eye distinction between the 2 types, is to be preferred, although our own attempts to devise such a method have so far been unsuccessful.

SUMMARY

1. Over 100 strains of oral lactobacilli have been isolated from carious teeth and examined by several methods, chiefly to determine their acidogenic powers.

2. On the basis of acid production from glucose, these strains have been grouped as follows: (a) S.A., those which produce a large quantity of acid. (b) W.A., those which produce a much smaller quantity.

3. This grouping is well supported by other biochemical properties such as (a) fermentation reactions, particularly with raffinose, salicin and mannitol and (b) ability to grow on a medium containing sodium taurocholate.

4. From a comparison of our results with those of Enright, Friesell and Trescher it would appear that our S.A. type corresponds to their Group I, and our W.A. type to their Group II, which groups they identify with Rettger and Horton's Y and X types respectively.

REFERENCES TO LITERATURE

ANDERSON, T. A. AND RETTGER, L. F., *J. D. Res.*, **16**: 489, 1937.
BUNTING, R. W., *Berichte 9. internat. Zahnarztekongresses der F.D.I.* Wien, **1**: 323, 1936.
CURRAN, H. R., ROGERS, L. A. AND WHITTIER, E. O., *J. Bact.*, **25**: 595, 1933.
ENRIGHT, J. J., FRIESELL, H. E. AND TRESCHER, M. O., *J. D. Res.*, **12**: 759, 1932.
FROST, W. D. AND HANKINSON, H., *Lactobacillus acidophilus*, (Wisconsin: Davis-Greene Corporation, 1931), p. 120.
HADLEY, F. P., BUNTING, R. W. AND DELVES, E. A., *J. A. D. A.*, **17**: 2041, 1930.
HOWE, P. R. AND HATCH, R. E., *J. Med. Res.*, **36**: 481, 1917.
KULP, W., *Science*, **66**: 512, 1927.
KULP, W. AND WHITE, V., *Science*, **76**: 17, 1932.
LONGWORTH, L. G. AND MCINNES D. A., *J. Bact.*, **29**: 595, 1935.
MCINTOSH, J., JAMES, W. W. AND LAZARUS-BARLOW, P., *Brit. J. Exp. Path.*, **3**: 138, 1922. *Idem*, *ibid.*, **5**: 175, 1924.
MORISHITA, T., *Proc. Soc. Exper. Biol. Med.*, **25**: 654, 1929.
RETTGER, L. F. AND HORTON, G. D., *Zbl. Bkt.*, Abt. 1., Orig., **73**: 362, 1914.
RODRIGUEZ, F. E., *Military D. J.*, **5**: 199, 1922. (Abstract only available).
ROSEBURY, T., *J. Bact.*, **24**: 321, 1932.
SULLIVAN, H. R., *J. D. Res.*, **18**: 525, 1939.
TOPLEY, W. W. C. AND WILSON, G. S., *The Principles of Bacteriology and Immunity* (London, 1936) p. 580.
ULICNY, H. P., *J. D. Res.*, **15**: 397, 1936.
WADSWORTH, A. B., *Standard Methods*. (Baltimore, William Wood, 1927.)
WHITE, B. AND AVERY, O. T., *Zbl. Bkt.*, Abt. 11., **25**: 161, 1910.

A COMPARISON OF THE DECALCIFYING ABILITY OF DIFFERENT TYPES OF ORAL LACTOBACILLI[1]

H. R. SULLIVAN

From the Department of Bacteriology, University of Sydney, Australia

It has been shown that oral lactobacilli isolated from carious teeth were, in general, either strongly acidogenic (S.A.), or weakly acidogenic (W.A.).[2] It was therefore decided to observe whether these 2 types of bacteria showed a corresponding difference when brought into contact with the enamel surface of sound human teeth.

Extracted teeth (incisors only) were examined by naked eye, under a dissecting microscope and radiographically, and areas of enamel, judged by these methods to be normal, were marked. Areas were usually chosen on approximal surfaces and measured approximately 2 mm. In order to facilitate handling, the root canals of the teeth were enlarged and the teeth mounted on wooden applicators. They were then sterilized in test tubes by autoclaving at 10 lbs. pressure for 10 minutes. Nutrient agar containing 1 per cent added glucose was prepared and after melting was cooled to 45°C. Suspensions of the organisms to be tested were then mixed into small quantities of this agar, and a thin layer of this mixture applied to the chosen area of enamel by means of a sterile platinum loop, and allowed to solidify. In this way it was possible to bring a small portion of enamel into relatively prolonged and close contact with the products of metabolism of selected strains of bacteria. The teeth bearing these "artificial plaques" were then suspended in 1 per cent glucose broth (pH 7.0) and incubated at 37°C.

In order to keep the environment of the teeth fairly constant, or at least to avoid any great accumulation of acids, the teeth were carefully transferred to fresh tubes of glucose broth twice daily, and were placed in ordinary broth (no added glucose) overnight. As controls, similar experiments were set up, where the "plaques" contained no organisms. After incubation under these conditions for 2 weeks, the teeth were washed thoroughly and again examined by the 3 methods mentioned above. Ground sections were prepared when necessary.

This procedure enabled control of the reaction of the surrounding medium and the amount and type of fermentable carbohydrate in the medium without disturbing the nidus in which the bacteria and presumably their metabolites were sheltered. Nine strains of each type of lactobacillus (i.e. our S.A. & W.A. types) were tested and 3 control teeth were used—21 teeth in all.

---

[1] This work was carried out under a grant from the National Health and Medical Research Council of Australia.

[2] Sullivan, H. R., Still, J. L., and Goldworthy, N. E., *J. D. Res.*, **18**: 513, 1939.

RESULTS

The 3 control teeth were found to have retained their original lustre, whilst the 9 teeth bearing plaques containing the weakly acidogenic (W.A.) organisms showed a definite dullness over the area that had been covered by the "plaque." The enamel still retained its former hardness however, and none of the usual signs of decalcification could be detected. The areas covered by the "plaques" containing the strongly acidogenic (S.A.) organisms presented a very different picture. The enamel had become a cream colour, and was quite soft and pitted. The fact that decalcification of the enamel had occurred within these areas was confirmed radiographically and by ground sections.

The area of decalcification thus obtained differs to a certain extent from that seen in a ground section of a naturally occurring initial carious lesion, but it must be remembered that, in order to retain the artificial plaque in position, the area covered had to be larger than one would normally find, thus giving greater opportunities for superficial decalcification. The centre of the decalcified area however, showed a typical cone shaped zone of altered enamel substance.

The main advantage of this method is that one can, to some extent, restrict the activities of the organisms to a small area where their physiological reactions can proceed undisturbed, whilst the remaining enamel is bathed in a fluid medium maintained at a comparatively constant level of H^+ ion concentration which is harmless to the enamel.

ANACORIC EFFECT OF CHRONIC PERIAPICAL INFLAMMATIONS

JULIUS CSERNYEI, M.D.

Milan, Italy

Circumscript chronic periapical inflammations and dental granuloma have long been considered as foci for various diseases. As the root canal had been considered, almost exclusively, the portal entry of the infection the importance of haematogenous infection was overlooked.

Menkin (1, 2, 3, 4, 5, 6, 7) observed that electronegative substances, such as trypan blue, colloidal iron and microorganisms, collect and become fixed in the inflamed tissues. Trypan blue, injected into the blood stream, quickly reaches the inflamed area. When injected into the area of inflammation, it is retained there and does not reach the regional lymph nodes. Colloidal iron, *B. prodigiosus* and *B. pyocyaneus*, injected intravenously, also reach the inflamed zone in a short time, the presence of iron having been demonstrated by qualitative and quantitative analysis.

Bareggi, Nai and A. Ascoli (8, 9, 10) observed, in calves inoculated with Calmette-Guerain weakened tubercle bacilli, a return to the acute stage in tuberculosis granuloma formed at the point of inoculation in endemic septicemia and other cases. The inoculated calves lived while those not inoculated died. The bacteria were found only in granuloma. When tuberculosis granulomas were excised, the animals had no resistance to the infection and died from septicemia. Animals to which the granulomas were transplanted, demonstrated similar resistance to those that were inoculated. It seems that zones of circumscribed inflammation attracts bacteria from the circulation and keep at least large parts of them alive for a long time. Ascoli calls this phenomenon *anacoresis* (gr. *αναχωρηδις*—refuge and also convocation). When the convocated bacteria have a morbigenous effect on the inflamed tissue they can produce local disturbances or reenter the circulation.

After removal of the pulp, a reaction begins in the periodontal tissues which ends in about 2 months (11). The periapical area will present a normal histologic appearance at that time if the extirpation was performed with asepsis and if no irritating antiseptics or foreign bodies were placed in contact with the periapical tissues; otherwise the inflammation around the root end will be chronic and persistent. Necrosis of the pulp in carious teeth is followed by the interruption of nutrition of the apical cementum which becomes necrobiotic or necrotic. This root end necrosis provokes the formation of, "demarcation granulation." The immigration of microorganisms complicates the process and, according to their virulence, leads to the formation of acute or chronic painless abscess. The demarcation granulation consists of activated mesenchymal cells and, in part, migrated blood elements. This inflammatory new tissue formation after a time will be incapsulated by connective tissue fibers to form the dental granuloma. Hyperemic tissue can be observed, attached to the root end even on teeth where encapsulation could not have occurred. Not only does the apical parodontium, in its strict sense, change in character but even in the marrow spaces of the bone granulation tissue can be observed.

METHOD

In order to find out if chronic periapical inflammations have an ancoric effect, the pulps were removed from the teeth of dogs and the cavity and pulp left open to provoke chronic periapical inflammation. After some weeks the dogs were injected in the vena saphena with a culture of Bang bacilli. These bacilli were used to eliminate possibility of natural infection. Three weeks after injection of the Bang bacilli the pulpless teeth and certain normal teeth were extracted, the root ends separated with sterilized cutting pliers, caught in sterile test tubes and then placed in sterile mortars with physiologic NaCl solution to separate the adhering tissues and microorganisms. The suspensions obtained were injected into guinea pigs. After 3 to 4 weeks the guinea pigs were killed and an agglutination test made with their blood sera against pure Bang bacilli cultures. Blood was taken from the hearts of the animals with Pasteur pipettes and injected in guinea pigs for agglutination tests. The autopsied dogs did not present any alterations in other organs that might have been attributed to Bang bacilli.

The guinea pigs were examined at autopsy and those injected with suspensions from the periapical inflamed tissues showed characteristic enlargements of the spleen with granular surfaces and elevated follicles. Serum of their blood agglutinated Bang bacilli. The spleens of animals injected with suspensions of the periapical tissues from

sound teeth and those with heart blood from the dogs gave negative results. The biological test method was used because if the Bang bacilli are not in a pure culture they degenerate so as to be difficult to identify. The results of the last 2 experiments are shown in Table I.

TABLE I

Results of agglutination tests on guinea pigs

	MATERIAL INJECTED	BLED TO DEATH ON (DAY)	AUTOPSY FINDINGS	RESULT OF AGGLUTINATION TESTS
I	Soft tissue suspension from periapical area of infected tooth 1, dog 1	47th	Splenomegaly	Till 1:400
II	Same as I	D*	Enteritis adenopathy	Not made
III	Soft tissue suspension from periapical area of infected tooth 2, dog 1	D	Splenomegaly adenopathy	Not made
IV	Same as III	47th	Splenomegaly	Till 1:400
V	Periapical soft tissue suspension from control tooth, dog 1	47th	Normal	Negative
VI	Same as V	D	Enteritis	Not made
VII	Heart's blood (dog 2)	47th	Normal	Negative
VIII	Same as VII	D	Enteritis	Not made
IX	Periapical soft tissue from infected tooth 1, dog 2	36th	Splenomegaly	Till 1:400
X	Periapical soft tissue from infected tooth 2, dog 2	36th	Splenomegaly	Till 1:200
XI	Periapical soft tissue from control tooth, dog 2	15th	Normal	Negative
XII	Same as XI	15th	Normal	Negative
XIII	Same as XI	30th	Normal	Negative
XIV	Same as XI	37th	Normal	Negative

* D, died before completion of experiment.

DISCUSSION

These experiments indicate that chronic periapical inflammations have an anacoric effect on microorganisms and that organisms do not remain in the blood but take refuge in the area of inflammation. The periapical area of sound teeth may remain uneffected by hematogenous infection. In one of the preliminary experiments, not cited, it was possible to obtain a pure culture of Bang bacilli from the root end

of a pulpless tooth. It can be assumed that periapical inflammations have the same effect on pyogenic and other bacteria in the blood which is known to be seldom sterile but to support a subclinical septicemia.

Treatment of teeth after devitalization should be carried out in such a manner as to prevent chronic periapical inflammation. The removal of the pulp must be executed with surgical asepsis. Equal parts arsenous and tannic acids mixed into a paste with creosote and applied directly on the pulp has been used by the author to sterilize the pulp and pulp cavity. Aseptic removal of the pulp protects the pulp cavity from secondary infection and hermetic sealing of the pulp chamber only, leaving the rest of the root canal unfilled, protects the periodontal tissues from irritation by foreign bodies and antiseptics (11). Foreign bodies and corrosive antiseptics used in treating and filling root canals can provoke a chronic inflammation in the periapical tissues and make them by anacoresis, in a secondary manner, pathogenic foci.

The root ends of teeth with necrotic and putrefied pulps are surrounded by chronically inflamed and infected tissues which may receive bacteria hematogenously. Since the tissues remain chronically inflamed after antiseptic root canal therapy, by anacoresis they may become foci for reentry of bacteria and toxins into the blood stream even after immigration from root canal has ceased.

Some phenomena observed in daily practice can be explained by anacoresis. When one of two necrotic roots is removed the patient may return in a few days with periodontitic pains on the remaining root. Okell (12) found that after extraction of diseased teeth a streptococcal bacteremia is demonstrable in 75 per cent of the cases. This observation together with the anacoric effect of chronically inflamed periapical tissues explains this clinical observation. Another example is the formation of an abscess after traumatic pulp necrosis in a sound tooth. The necrotic pulp produces a disturbance of metabolism in the periapical tissues which respond with inflammation. The inflamed tissues attract pyogenic microorganisms, not all of which can be phagocytized and an abscess results.

When anacoresis is recognized as proven we may also view "oral sepsis" from a different viewpoint. Rosenow and others (13, 14) have shown that microorganisms, introduced into root canals, can be

found in other diseased organs. It may be that microorganisms from a diseased organ migrate to the periapical areas of pulpless teeth. It is not certain whether the organisms found in any peridental focus have migrated from the root canal or have come through the blood stream. There seem to be 2 phases in "elective localization"; in the first the bacterial toxins enter the circulation and disturb the metabolism of cells of certain organs, that have a specific sensitiveness and in the second the organism reacts to the disturbed metabolism with inflammation and attracts microorganisms from the primary focus. While this hypothesis may seem venturesome it is the desire of the author to direct attention to the possibility.

SUMMARY

Chronic periapical inflammation attracts microorganisms from the blood, the bacteria remaining alive. This phenomenon is anacoresis. Treatment of teeth with vital pulps should be carried out so as not to provoke chronic periapical inflammation or an infected focus can develop. Teeth with putrescent pulps always have a chronical inflammation around the root end, which may also become infected hematogenously. They must be treated to eliminate the foci by surgical intervention or extraction where other operations are not possible.

REFERENCES TO LITERATURE

1. Menkin, V., *J. Exp. Med.*, **50**: 171, 1929.
2. *Idem, ibid.*, **51**: 879, 1930.
3. *Idem, ibid.*, **52**: 201, 1930.
4. *Idem, ibid.*, **53**: 171, 1931.
5. *Idem, ibid.*, **53**: 179, 1931.
6. *Idem, ibid.*, **53**: 647, 1931.
7. Menkin, V., and Menkin, M., *J. Exp. Med.*, **51**: 285, 1930.
8. Bareggi, G., *Pat. Comp. Tbc.*, **2**: 218, 1937.
9. Ascoli, A. and Nai, D., *Pat. Comp. Tbc.*, **1**: 5, 1938.
10. Ascoli, A., *Pat. Comp. Tbc.*, **2**: 191, 1937.
11. Csernyei, J., *Zahn. Rdsch.*, **43**: 1250, 1933.
12. Okell, J., *Lancet*, Nov. 19, 1935.
13. Rosenow, E. C., *Ann. Clin. Med.*, **1**: 211, 1923.
14. *Idem, Verh. D. Ges. I. Med.*, **47**: 408, 1930.

A COMPARISON BETWEEN DIETARY AND HORMONAL FACTORS IN THE DEVELOPMENT OF DENTIN

GERRIT BEVELANDER AND MARGARET M. HOSKINS

Department of Anatomy, College of Dentistry, New York University

INTRODUCTION

From a study of the literature on the relation of Ca metabolism to hard tissues it is apparent that two factors, at least, are to be considered in the development of dentin and enamel. These are: first, the Ca content and Ca/P ratio of the diet; second, the amount of parathyroid hormone available to mediate the normal distribution of the dietary Ca and P throughout the body.

Erdheim (1) and Toyofuku (2) produced severe lesions in the incisors of rats by removing the parathyroids of these animals. Similar results have been obtained by a number of subsequent investigators (3, 4, 5), so that it is now well established that parathyroidectomy is followed by abnormalities in the dentin laid down after operation. The diets used by the different investigators have apparently affected the results considerably, but data as to the mineral content of the rations are not available.

In the present study we have undertaken a comparison of the effects of dietary and hormonal factors on the development of dentin. In two series we have observed the effects of variation in the Ca content and the Ca/P ratio of the diet. In another, lesions were produced by parathyroidectomy alone, the mineral content of the diet being kept normal, while in the fourth group parathyroidectomy was combined with a marked dietary Ca deficiency. In all groups, we have attempted to correlate the effect produced on the dentin with that other well known symptom of disturbed Ca metabolism, the serum-Ca level.

MATERIALS AND METHODS

The animals used in this study were albino rats of the Wistar strain. With the exception of one group they were all sexually mature and 90 days old when experimentation

began. The animals were placed on a known diet for 1 week. At the end of this time, a number were parathyroidectomized; others were reserved for control. After operation, madder was added to the diet of both the operated and the control rats until the time of killing. Controls were sacrificed at the same time as operated animals. One group of non-operated animals, 30 days old, was fed a rachitogenic diet for 21 days. At the end of this time they were autopsied. Three diets were fed in the experiments which are to be described: 1) commercial Purina chow (Ralston), 2) diet E (6) and 3) the Steenbock diet No. 2965. The Ca/P ratios of these diets were 2:1, Ca free, and 4:1, respectively.

At the termination of the test period, blood was drawn by cardiac puncture, while the animals were under anesthesia, and set aside to be tested for serum calcium by the Clark-Collip (7) modification of the Kramer-Tisdal method. The animals were then killed and the teeth were preserved in suitable fixing fluids. Both ground (8) and decalcified sections of the teeth were prepared and examined.

Neck regions were preserved for serial sectioning in the event that incomplete removal or the presence of accessory parathyroid material was suspected. This check (9) on parathyroidectomy has already been shown to be reliable.

OBSERVATIONS

I. EFFECTS ON DENTIN

A. Dietary Effects

1. Control chow. Control animals fed on chow diet developed normally in every respect insofar as we were able to ascertain.

2. E Control. Some of the teeth in the E control series showed abnormal development of dentin. The maximum extent to which these abnormalities developed is shown in *fig. 7*, a typical example of this condition. Reference to this figure shows that the disturbance in the formation of dentin starts rather slowly. At first there is no change in appearance at all; then abnormally wide incremental bands appear, and finally a wide zone of interglobular dentin is formed. This seems to indicate a disturbance in calcification which starts rather slowly and becomes progressively more severe in character with the passage of time. A second variation in the formation of dentin in this series also occurs. In these cases we observed an accentuation in the width, irregularity, and staining qualities of the incremental lines. Other teeth examined in this series appear to be normal in respect to the formation and structure of the dentin which was laid down during the test period.

3. Steenbock diet. Pronounced alterations in the formation of the dentin result when rats are fed the Steenbock diet. This is clearly shown by *fig. 9*, a section of an incisor showing the typical condition

of this disturbance. The first indication of a change from the normal appearance of dentin occurs as an accentuation in the width of incremental lines. In ground sections this condition can be observed as a light zone. The region of accentuated banding is then followed by a wide band of interglobular dentin. From that point, the dentin is seen to be laid down in alternating zones in which the tissue is by turns nearly normal, or defective in either of the two ways just described. This condition persisted for the entire observation period of 24 days.

B. Operative Effects

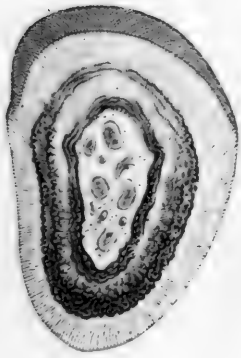
1. Chow diet. The effects of the removal of the parathyroid glands on the development of dentin are in many respects similar to those just described. At first one or more bands appear which stain intensely with haematoxylin. After this a wide zone of interglobular areas occurs. These are the immediate responses to the removal of the glands. The subsequent formation of dentin is subject to a number of variations in different teeth and in different parts of the same tooth, as shown by the *figs. 1–6* in Plate I. In the portions of the tooth in which these disturbances persist, however, as revealed by the examination of teeth obtained from animals which were killed 30 days after operation, the dentin is laid down in alternating zones, some of which are normal and others defective, as described above (*figs. 10, 13 and 14*). The continued development of dentin was examined in groups of animals which were sacrificed 60 and 90 days, respectively, after operation. Examination of the teeth of the 60 day specimens shows that abnormal production of dentin still persists, but to a degree somewhat less severe than that which occurs in the 30 day specimens. This typical condition is clearly shown in *fig. 11*. Examination of this section shows that the peripheral dentin is still interglobular in character whereas the dentin which lies toward the pulpal surface is more uniform. *Fig. 12*, represents a similar section of the typical condition which occurs in a 90 day post-operative specimen. After the lapse of this period, the disturbance in the production of dentin is even less pronounced than in the 30 or 60 day specimens referred to above.

2. Diet E. The second group of operated animals in which the development of the tooth was studied differed from the first group in that they were fed a Ca-deficient diet (diet E). This group consisted

1.

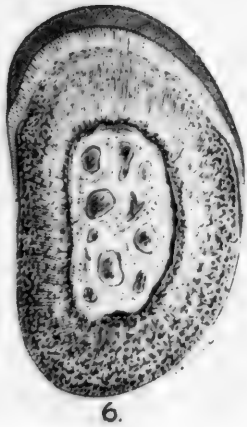
2.

3.



4.

5.



6.

PLATE I

of 24 animals which were killed 24 days after operation. The initial effects on the dentin in these animals were in most respects similar to those described for the chow series; i.e., the appearance of wide irregular incremental lines and the formation of a wide interglobular zone. Certain characteristic differences in the structure of dentin, however, appeared in this group. These differences were as follows: 1) The initial lesion occurred more rapidly and was more intense in character; i.e., the interglobular area was wider and less normal than that previously described. This is well shown by referring to *figs. 8 and 15*. 2) The dentin which was formed after the initial response is of an entirely different character from that described for the chow series. Instead of the alternating bands of normal and abnormal dentin characterized by the large interglobular areas and intensely staining

PLATE I

Figs. 1–6 are drawings made from transverse ground sections of lower incisor of rat which was parathyroidectomized and fed on chow diet. Autopsy took place 30 days after removal of glands. Orig. mag. 25×. Tooth was arbitrarily spaced into 6 equal divisions from incisal surface to the anterior surface of the third molar. The sections represent levels at 6 equidistant points between these 2 regions. Sections show effect of parathyroidectomy on subsequent development and rhythmical deposition of dentin, abnormal structure of dentin which develops post-operatively and amount of tissue which is involved after 30 days of development.

incremental lines, this tissue appears to be predentin, showing practically no sign of calcification.

In the foregoing pages observations are presented which form a basis for comparison of the effects produced on dentin by various dietary and hormonal alterations in the mineral metabolism of the rat. It should be emphasized at this point that the process under consideration is the formation of new dentin in the constantly growing tooth. The old dentin formed before the beginning of the experiments did not show any effect which could be attributed to the experimental treatment.

A low Ca content and Ca/P ratio, such as diet E provides, gives rise to abnormalities in the newly formed dentin even though the parathyroids of the animal in question are not removed. The effects produced are variable. Some teeth may be entirely normal; others may show intensification of incremental banding; and still others may

exhibit enlargement of interglobular areas. It is of interest in this connection to note that when abnormalities occur, they are diffused throughout the dentin formed during the test period, and present no evidence of an initial violent disturbance from which there is a gradual recovery.

The teeth of the unoperated animals which were fed the Steenbock diet, Ca/P 4:1, and the teeth of those which were parathyroidec-

PLATE II

The figures in Plate II are drawings of ground sections of lower incisors of rats subjected to various experimental treatment. These sections were cut at level of second molar. Orig. magnification 80×.

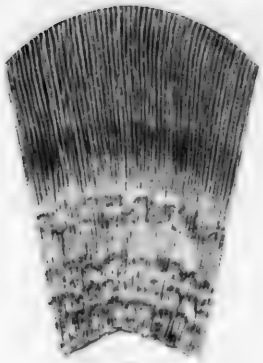
Fig. 7. Section of lower incisor of non-operated animal fed diet E for 24 days. Note that dentin nearest pulp cavity shows greatest amount of interglobular areas.

Fig. 8. Section of incisor of rat which was parathyroidectomized and fed diet E (low Ca). This section shows wide band of hypo-calcified dentin, then wide zone of interglobular areas, and finally wide zone of pre-dentin.

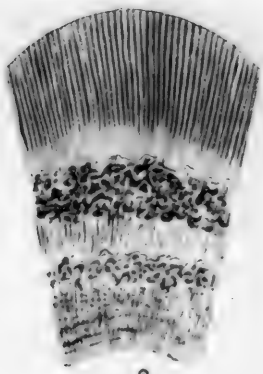
Figs. 9, 10, 11 represent sections of teeth obtained from specimens which were parathyroidectomized and fed on chow. *Fig. 9* shows the structure of the dentin in a rat which was killed 30 days after operation. Note that at this level there is a fairly wide zone of normal preoperative dentin. Passing pulpward one sees banding effect; next zone of interglobular areas, then a zone of dentin in which direction of tubules is distorted. This disturbance in formation of dentin is repeated several times. *Fig. 9* represents a section of tooth of an animal which was autopsied 60 days after operation. At this level, the entire dentin is postoperative. Note that while the peripheral dentin is still subject to disturbances in metabolism which cause formation of interglobular areas, remainder is less subject to these disturbances. *Fig. 10* shows the structure of dentin in tooth of animal killed 90 days after operation. The dentin in this tooth shows again tendency for the structure of this tissue to develop in direction tending towards normal condition.

Fig. 12. Section of incisor of non-operated rat fed Steenbock diet 2965 for 21 days. Note that structure of dentin in this tooth is in most respects similar to that of the parathyroidectomized chow-fed animals; e.g., accentuation of incremental lines, interglobular zones, and dentinal tubules present in distorted forms. This disturbance is repeated several times, as examination of this figure clearly shows.

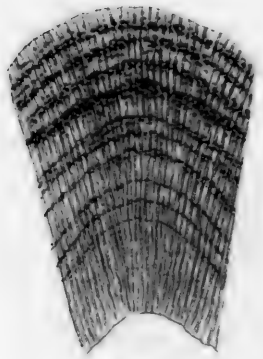
tomized and fed on chow (Ca/P, 2:1) are much alike. In both groups we find the following conditions: a zone of intense calcification is followed by one of large interglobular areas; from there on the dentin is seen to be laid down in alternating zones in which the tissue is by turns nearly normal and filled with interglobular areas. The proportion of the two zones does not, however, remain constant in the dentin of parathyroidectomized animals. It is evident as time passes that the deposition of dentin tends towards a normal course, as is shown by



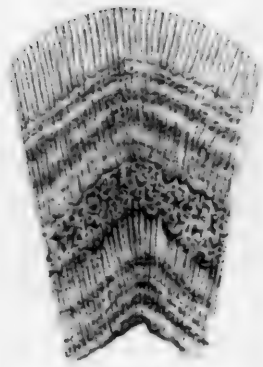
7.



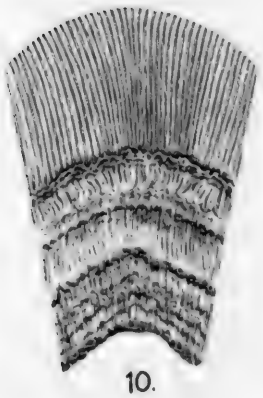
8.



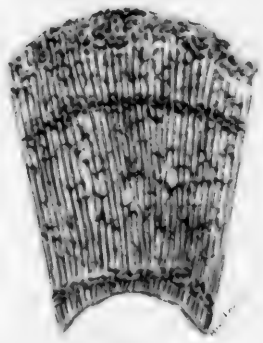
12.



9.



10.



11.

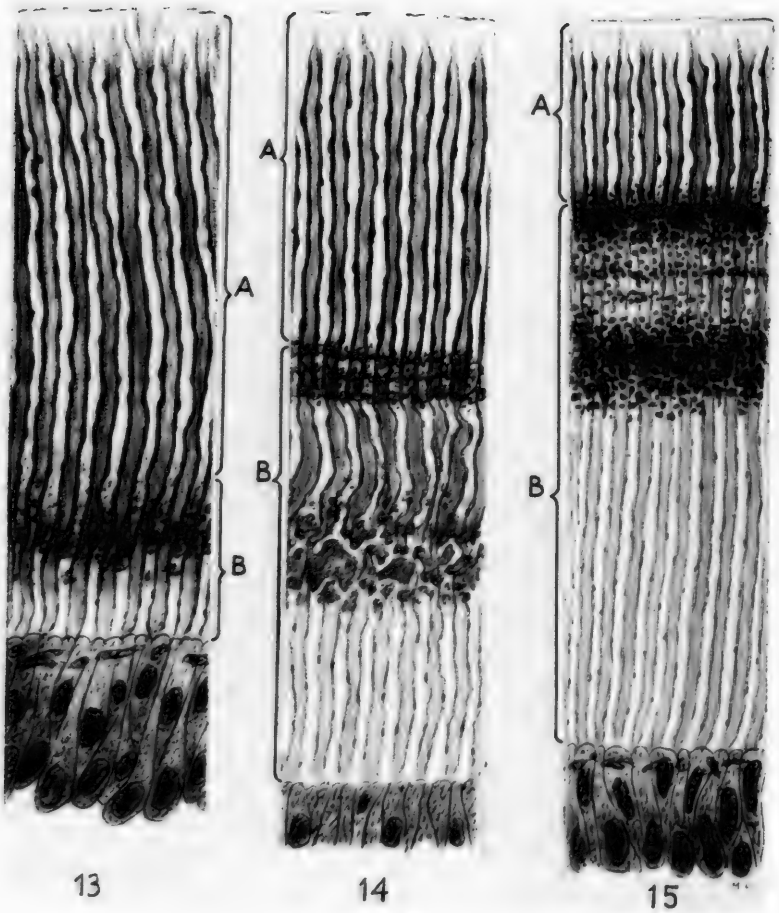


PLATE III

examination of the tissue in teeth of specimens killed 60 and 90 days after operation. In the teeth of these animals the zones of normal dentin become wider and the bands of interglobular dentin become correspondingly reduced. It is possible that were the animals allowed to live for a longer time, normal conditions might have been established in the dentin.

Parathyroidectomy followed by dietary Ca deficiency produces severe initial lesions which are followed by the production of embryonic

PLATE III

Figs. 13, 14 and 15 are drawings of decalcified sections of lower incisor of rat. These sections were cut at level of molar 1–2 and stained with hematoxylin and triosin. Orig. magnification 250×. *Fig. 13* represents section of control animal fed chow diet. Age at autopsy 120 days. A, represents well calcified mature dentin, B, less highly calcified zone known as pre-dentin. In latter zone calcified material forms typical globules which gradually coalesce as this tissue moves in a peripheral direction. Compare these two zones with those shown in *figs. 13 and 14*. *Fig. 14*. This figure shows a section of lower incisor of specimen which was parathyroidectomized and fed on chow diet. Autopsy was performed 30 days after operation. A, pre-operative, normal dentin; B, post-operative dentin. Note that in this section accentuation of incremental lines is clearly shown. These areas stain intensely with hematoxylin. From this point dentin is abnormal, as shown by presence of interglobular areas and tortuous dentinal tubules. These details are clearly shown in this figure. *Fig. 15* represents structure of dentin observed in specimens which were parathyroidectomized and fed diet E (low Ca). A, represents normal pre-operative dentin; B, post-operative dentin. In comparing the postoperative dentin shown in *fig. 14* with that shown in this figure, it is evident that the initial disturbance is more intense in latter. Incremental lines are wide and stain heavily with hematoxylin. The remainder of the post-operative tissue shows some dentin which is interglobular in character, but the greater part of this zone is distinctly pre-dentin, showing no evidence of calcification whatever.

non-calcified dentin. The dentin of these animals shows no tendency to return to a normal condition.

II. COMPARISON OF DENTIN AND SERUM CALCIUM

The serum calcium relationships which were observed in the various groups of animals were as follows: In the chow control series, the figures were observed to be approximately 10 mg. per cent Ca per 100 cc. of blood. As previously stated, the dentin which developed in these animals was normal in every respect insofar as we were able to judge.

In the diet E controls, serum-Ca values were observed to vary from

7.8 mg. per cent–10.1 mg. per cent. The structure of the dentin which developed during the test period also varied in different animals from a condition apparently normal to that in which either accentuated incremental bands or interglobular areas appeared. There was, however, no correlation between either of these histological conditions and the serum-Ca values.

One specimen, for example, which was observed to have a serum-Ca of 10 mg. per cent had defects much more pronounced than another in which the serum-Ca value was 7.8 mg. per cent.

Animals which were fed the Steenbock diet were observed, when tested, to have a relatively high serum-Ca, the values averaging 12.3 mg. per cent for 18 animals. In this group, however, marked disturbances in the rhythmic production of dentin were seen, as previously described, despite the high serum-Ca values. The serum-Ca values of the parathyroidectomized animals which were fed the chow diet were observed to drop from 10 mg. to 7.5 mg. per cent within 24 hours after operation. Determinations made at increasingly longer post-operative intervals were ascertained to be: 6 da., 7.7; 12 da., 7.5; 24 da., 6.6; 30 da., 7.2; 90 da., 6.7 mg. per cent, respectively.

In a series of 60 animals which was fed chow diet and which was autopsied thirty days after operation, an attempt was made to correlate variations in the structure of dentin following parathyroidectomy with observed differences in the serum-Ca values. The condition in two animals which typifies these relationships is as follows: In one animal the Ca was 6.8 mg. per cent, in the other, it was 8.0 mg. per cent. The severity of the disturbance in the formation of dentin was greater in the latter than in the former. Many cases of the opposite relationships were also observed. From our study of 60 cases we were unable to ascertain any degree of either positive or negative correlation between the severity of disturbance in developing dentin and the observed serum-Ca values.

Operated animals which were fed diet E were observed to have serum calcium values which averaged for the group 5.2 mg. per cent. In this series of animals there were at least two sets of factors which were operating to prevent the normal production of dentin: 1) low calcium intake and 2) absence of parathyroid glands. Under these conditions the dentin which developed after operation was consistently embryonic

in character; i.e., hypocalcified, and did not at any time show a tendency to revert to the normal condition such as we observed in operated animals fed the chow diet.

The data referred to above are represented graphically in *text-fig. 1.* Reference to this figure clearly shows the consistent differences in serum calcium values obtained among the several groups which

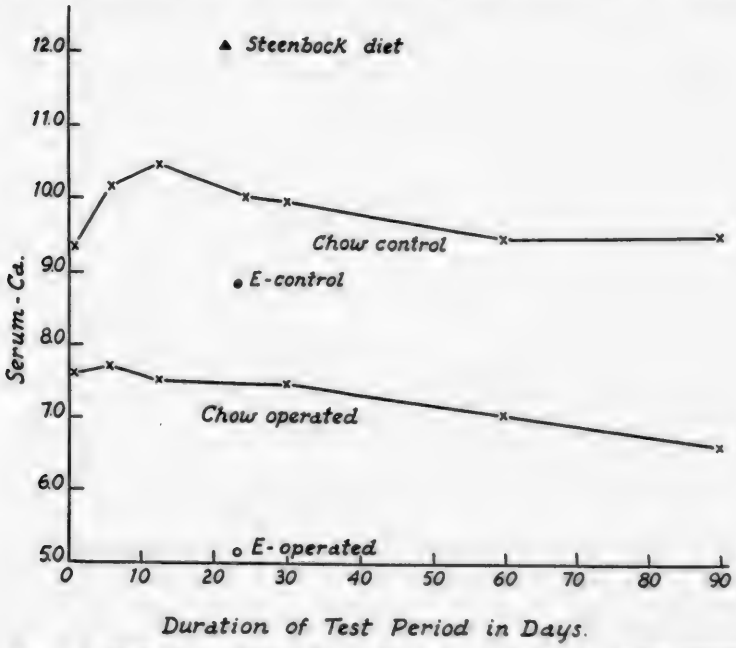


FIG. 1. Relation between serum-Ca, diet and parathyroidectomy. Points represent the average values for determinations made in each group consisting of 18, 30, 120 animals fed the Steenbock, E, and chow diets respectively.

were examined. In addition, it shows the various time relationships which were taken into account in making these observations.

DISCUSSION

1. DIET, PARATHYROIDS, AND DENTIN

Diet E is very nearly calcium free. Animals on this ration maintain a serum calcium level which is little below the normal figure, so

long as their parathyroids are intact. They do so, however, in some cases at the expense of the calcification of new dentin. Unpublished results obtained by Krimsky (10) in this laboratory show that the long bones of these animals suffer in the same way. New bone is laid down as osteoid tissue deficient in calcium salts. In both bones and teeth, tissue laid down before the beginning of the experiment remains normal; the condition is one in which calcium is withheld from new tissue rather than withdrawn from previously formed tissues. In this situation of calcium starvation there is no evidence that the structure of the dentin improves with the passing of time. The lesions are produced by a deficiency of the required material and no effort on the part of the parathyroid can make up for such deficiency.

The results obtained by feeding animals on a rachitogenic diet are similar to those of parathyroidectomy. Both may be considered as examples of faulty distribution of Ca and P in the body. In the case of the rachitic rats the origin of this disturbance is the abnormal Ca/P ratio of the diet (4:1 instead of 2:1). The insufficient P in proportion to the Ca does not permit the normal deposition of calcium phosphate in the dentin, and an excess of Ca remains in the serum.

In the parathyroidectomized rat the organ responsible for the proper distribution of the mineral elements is missing. Here, if the animals are on an adequate diet, such as Purina chow, adequate Ca and P are available in the normal proportions. The mechanism for maintaining the elements in circulation and for depositing them in the dentin is, however, wanting, and calcification of dentin and serum-calcium level are both disturbed. In parathyroidectomized animals the tendency towards improvement of the dentin, observed in the latter part of the test period, must be attributed to some compensatory mechanism, such as that postulated by Hoskins (11) to explain the decrease in susceptibility to tetany which occurs in post-operative survivors.

In parathyroidectomized rats on diet E there is, obviously, a more severe disturbance of Ca and P metabolism than in any of the other experimental animals examined in this study. This disturbance consists, on the one hand, of removal of the gland which normally mediates the metabolism of these elements, and on the other, of a marked deficiency of the elements themselves. In this situation the compensatory mechanism has practically no Ca with which to work, and the deposition of salts in the dentin is impossible.

2. SERUM-Ca AND DENTIN FORMATION

Comparison of the data on serum-Ca with the condition of the teeth in different groups of animals gives rise to the strong impression that calcification of dentin is not causally related to the serum-Ca level. It is true, taking the average figure for blood Ca and the most commonly observed dentinal conditions in certain groups, that a series may be arranged which will suggest that the two results of the treatment are correlated. For instance, disturbances in calcification of dentin are least noticeable among the control animals on diet E, in which group the serum-Ca figures average 8.9 mg. per cent. Next in order of severity of dentinal lesions are the parathyroidectomized animals fed on chow; these animals had an average serum-Ca of 7.5 mg. per cent. The most marked absence of calcification was observed in the group which were parathyroidectomized and fed diet E, and in which the average of the serum-Ca fell to 5.2 mg. per cent.

This arrangement of data is, however, upset by the inclusion of the cases fed on the Steenbock diet, in which the blood-Ca was 12.3 mg. per cent, but in which the dentinal calcification was disturbed. Other considerations, moreover, tend to weaken the case for a strict correlation between the extent of dentinal lesions and the serum-Ca level. The first of these is the variations within the groups. For instance, among the unoperated animals on diet E, in which the blood-Ca figures range from 7.2 mg. per cent to 10.1 mg. per cent, the condition of the dentin may be normal or abnormal. There is no evidence of correlation in individual cases between the two expressions of disturbed Ca metabolism. On the contrary, some of the most abnormal dentin in the group was found in animals with a normal serum-Ca level.

Another indication of the partial independence of serum-Ca and dentinal calcification may be seen in the history of parathyroidectomized rats fed on chow diet. In these animals the serum-Ca dropped to its low level of 7.5 mg. per cent within the first 24 hours after the operation—the period during which the most severe disturbances occurred. But while in the course of 90 days of post-operative life, dentinal calcification showed a gradual improvement, serum-Ca values remained at the 24-hour level, showing no consistent tendency to return to the normal figure.

It appears, therefore, from the data we have collected, that ab-

normal serum-Ca and faulty calcification of dentin are alike, both being symptoms of disturbances in the Ca and P metabolism of the body. Of the two, dentinal calcification seems to be the more sensitive indicator of such disturbances, since it responds to dietary deficiencies which have little or no effect on the serum-Ca.

We can summarize the relation between Ca intake, parathyroidectomy, and the disturbances produced in the development of dentin as follows:

	SEVERITY OF DISTURBANCES IN DENTIN	DURATION OF EFFECT ON DENTIN
1. Variation in Ca intake (Ca low)	+	Permanent
2. Variation in Ca/P ratio, Ca/P 4:1	++	Permanent
3. Variation in Ca/P distribution (parathyroidectomy)	++	Reverts to normal
4. Variation in distribution (parathyroidectomy) plus Ca deficient diet	+++	Permanent

REFERENCES TO LITERATURE

1. Erdheim, J., *Frankfurt. Ztschr. f. Path.*, **7**: 295, 1911.
2. Toyofuku, T., *Frankfurt. Ztschr. f. Path.*, **7**: 249, 1911.
3. Von Spreeter, T., *Deutsche Zahn-, Mund-, und Kiefer-Zahnh.*, **3**: 318, 1936.
4. Korenchevsky, V., *J. Path. and Bacteriol.*, **25**: 366, 1922.
5. Schour, I., S. B. Chandler, and W. R. Tweedy, *Amer. J. Path.*, **13**: 945, 1937.
6. Hoskins, M. M., Endocrinology, **19**: 453, 1935.
7. Clark, E. P., and J. B. Collip, *J. Biol. Chem.*, **63**: 461, 1925.
8. Bevelander, G., *Anat. Rec.*, **69**: 333, 1937.
9. Hoskins, M. M., and S. B. Chandler, *Anat. Rec.*, **30**: 95, 1925.
10. Krimsky, I., *Unpublished results*, 1939.
11. Hoskins, M. M., *Endocrinology*, **15**: 324, 1931.

COMPOSITION OF PRECOLUMBIAN TEETH

Calcium, Phosphorus, and Carbon Dioxide Determinations on all the Dentin and all the Enamel[1]

ETHEL L. FRENCH, Ph.D., HELEN TEFFT, MARGARET RATHBUN, JANET ECKHARDT, EILEEN WELCH and HAROLD C. HODGE, Ph.D.

From the Department of Chemistry, College of Arts and Science, and the Department of Biochemistry and Pharmacology, School of Medicine and Dentistry, University of Rochester, Rochester, New York

INTRODUCTION

The analysis of teeth of the precolumbian Indian should afford a number of interesting comparisons with the values for modern teeth Primitive tribes are often described as superior to modern civilized peoples in their dental equipment and dental health. Does this superiority lie in chemical differences in tooth composition? Did the primitive dietary produce a different sort of calcified product? The literature contains, to our knowledge, only the analysis of a single whole tooth whose age is chronologically comparable. Therefore, it seemed valuable to undertake the analysis of a number of precolumbian Indian teeth, to determine whether the "remarkable constancy" of composition previously found for modern whole teeth, as well as for enamel and dentin separately, would also be established for precolumbian teeth. In brief, this proposition was shown to be true. Especially for calcium and phosphate contents, precolumbian teeth and modern teeth have nearly identical chemical compositions. Therefore, the superior dental health of the precolumbian indian cannot be attributed to major differences in the principal inorganic constituents. Furthermore, the analytical data seem to show that the principal inorganic molecule is hydroxylapatite, whose occurrence in modern teeth has been many times described (3), (14).

Thirty-two precolumbian teeth were furnished by Dr. Aleš Hrdlička of the U. S. National Museum, at whose suggestion the work was

[1] This work was supported in part by a grant from the Carnegie Corporation of New York.

undertaken. The teeth, taken from several mouths, were in a remarkable state of preservation; few were carious, but attrition was observed in a majority of the teeth.

The recent description of a rapid quantitative method for the separation of enamel and dentin (1) offers the opportunity for determinations of the inorganic constituents in the separated tissues. These data may be compared on the one hand with similar data on modern dentin and enamel (2, 3) and on the other with the few existing analyses of fossil teeth.

METHODS

The precolumbian teeth were classified as to the approximate age of the person, size and position of the tooth, and evidences of caries, pyorrhea and calculus. The teeth (3) were separated into the enamel and the dentin portions[2] according to the centrifugal flotation method (1). The organic matter was extracted by 3 per cent KOH in glycol (2). On these samples of dentin and enamel, Ca, P and CO_2 determinations were carried out by the following procedures. Dentin and enamel inorganic samples weighing 100–125 mg. were dissolved in 100 cc. of dilute HCl. Aliquots which furnished approximately 4 mg. of Ca were taken in duplicate for Ca determinations, using a modification of the Clark and Collip method (4) by LeFevre and Nicholson (5). The CaC_2O_4 precipitates were separated by filtration instead of by centrifugation. Phosphorus determinations were run in duplicate according to Holtz's method (6). Single CO_2 determinations were made using the Shear and Kramer method (7).

RESULTS

A detailed description of the precolumbian teeth tabulated according to tooth position is given in Table I. It is interesting to note that the teeth are in most cases heavier than the corresponding present day teeth (2). Twenty-two teeth came from Indians whose ages were estimated (by the amount of attrition and from the root formation) as between 20 and 35. Only 5 teeth showed any caries, while 26 teeth showed attrition, in many cases severe. Most of the teeth were discolored, many of them being a brownish yellow. Many of the crowns were marked with a variable number of black lines of unknown significance. Nine teeth showed evidence of considerable pyorrhea and calculus.

Dentin: Enamel: In Table II the percentages of dentin, of enamel and of loss obtained in the separation of the dry teeth are given.

[2] The teeth were thoroughly cleaned with a dental burr before enamel and dentin were separated for subsequent analyses.

TABLE I

*Description of the Precolumbian Teeth**

TOOTH						ROOT DENTIN					
No.	Position	Size[1]	APPROX. AGE	CARIES[2] CLASS	ATTRITION[3]	Consistency[4]	Lgth.[5]	Color[6]	PERIODONTAL RESORPTION[3]	CALCULUS[3]	GENERAL REMARKS
6	$\overline{1}$	L	10	0	0+	N	In	W	0	0	
8	$\underline{4}$	L	15	0	0	N	N	W	0	0	Gingival third hypoplastic
32	$\underline{4}$	M	25	0	2	N	L	P Y	0	2	
21	$\overline{7}$	S	35	0	2+	N	S		0+	3	
27	$\overline{8}$	M	40	1	2	C	S		0	2	
15	$\overline{1}$	M	20	0	1	N	N		0	0	Crown stained brownish black
28	$\overline{1}$	S	30	0	2+	N	S	P Y	4	1	Tooth lost prematurely? Some enamel gone
30	$\overline{3}$	M	35	0	2	N	N	D Y	0	0	
31	$\overline{4}$	M	20	0	0	N	N	P Y	0	1	
19	$\overline{6}$	M	60	0	4	N	N	D Y	1+	?	Gingival portion of enamel chipped off
20	$\overline{6}$	M	55	0	4+	C	S	W	4	1	
7	$\overline{7}$	M	35	0	3+	N	S	Y	0	0	
18	$\overline{7}$	M	35	0	4	C	S	V D Y	1	2	Hypoplastic groove in gingival portion of crown
29	$\overline{8}$	M	40	1	3	N	N	P Y	1	0	Some enamel gone
2	$\underline{1}$	L	30	0	3	N	N		0	0	Lingual pit
9	$\underline{1}$	L	30	0	2+	C	L	D G	0	0	Very black stain on crown
10	$\underline{2}$	M	25	0	2	I	N	Y	0	0	
1	$\underline{3}$	L	20	0	2	N	N		0	0	Open root canal
24	$\underline{5}$	M	30	0	1	C	S	V D Y	0	0	
13	$\underline{6}$	M	40	1	3+		N	D Y	4	0	
16	$\underline{6}$	M	55	0	4+	N	S		3	4	
22	$\underline{6}$	M	35	0	3	So	S	Y	0	0	
4	$\underline{7}$	L	40	0	4	P	N		2	2	Calculus-distal-buccal on the gingival
3	$\underline{8}$	M	30	0	0	N	S	W	0	0	Deep occlusal fissures on top of tooth
5	$\underline{8}$	M	30	0	0	N	L	W	0	4	Deep occlusal fissures on top of tooth; calculus on crown
11	$\underline{2}$	M	20	0	0		S	W	0	0	
17	$\underline{2}$	M	20	0	0	C	N	Y	0	0	
14	$\underline{3}$	M	30	0	2	N	N		0	0	
26	$\underline{3}$	M	25	0	2	C	S		0	0	
23	$\underline{5}$	L	20	0	0+	N	L	Y	0	4	
12	$\underline{6}$	M	45	1	4+		N	D Y	4	4	Large softened mesial cavity at gingival line in dentine
25	$\underline{6}$	M	35	3	3	N	N		2	4	Sl. softening of enamel at mesial contact, black stain on occlusal. Chewing habit?

* The authors are glad to acknowledge the assistance of Dr. Virgil D. Cheyne for the classification and description of the teeth.

[1] Size: L—long, M—medium, S—small or short.

[2] Caries class: 0—none, 1—slight, 3—severe.

[3] Attrition, Periodontal Resorption, Calculus: 0—none, 1—slight, 2—moderate, 3—marked, 4—severe.

[4] Consistency: N—normal, So—soft, C—chalky, I—irregular.

[5] Length: N—normal, In—incomplete, L—long, S—short.

[6] Color: G—gray, W—white, Y—yellow, P—pale, D—dark, V—very.

The average percentage of dentin (60.5) from all the teeth is approximately twice the average per cent of enamel (27.8). The average percentages of dentin and enamel for carious teeth (62.5 and 25.8

TABLE II

Part a. Amounts of Dentin and Enamel in the Dry Precolumbian Teeth

TOOTH			DENTIN	ENAMEL	LOSS	TOOTH			DENTIN	ENAMEL	LOSS
No.	Position	Caries				No.	Position	Caries			
			%	%	%				%	%	%
6	$\frac{1}{}$	0	66.91	24.83	8.26	10	$\frac{2}{}$	0	60.75	25.87	13.38
8	$\frac{4}{}$	0	52.28	36.06	11.66	1	$\frac{3}{}$	0	69.64	23.02	7.34
32	$\frac{4}{}$	0	71.52	20.85	7.63	24	$\frac{5}{}$	0	54.39	35.91	9.70
21	$\frac{7}{}$	0	51.37	36.21	12.42	13	$\frac{6}{}$	1	66.27	22.10	11.63
27	$\frac{8}{}$	1	46.45	42.96	10.59	16	$\frac{6}{}$	0	72.31	16.31	11.38
15	$\frac{1}{}$	0	66.29	20.93	12.78	22	$\frac{6}{}$	0	†	30.60	
28	$\frac{1}{}$	0	†	14.72*		4	$\frac{7}{}$	0	45.94	41.03	13.03
30	$\frac{3}{}$	0	68.53	20.38	11.09	3	$\frac{8}{}$	0	58.16	32.61	9.23
31	$\frac{4}{}$	0	70.06	20.33	9.61	5	$\frac{8}{}$	0	39.42	35.18	25.40
19	$\frac{6}{}$	0	69.23	21.95*	8.82	11	$\frac{2}{}$	0	66.34	20.53	13.13
20	$\frac{6}{}$	0	58.29	32.87	8.84	17	$\frac{2}{}$	0	60.36	24.51	15.13
7	$\frac{7}{}$	0	60.40	30.65	8.95	14	$\frac{3}{}$	0	†	12.14	
18	$\frac{7}{}$	0	55.28	33.77	10.95	26	$\frac{3}{}$	0	43.09	39.76	17.15
29	$\frac{8}{}$	1	64.49	28.60*	6.91	23	$\frac{5}{}$	0	64.80	26.99	8.21
2	$\frac{1}{}$	0	62.55	26.27	11.18	12	$\frac{6}{}$	1	69.76	8.79	21.45
9	$\frac{1}{}$	0	56.06	28.72	15.22	25	$\frac{6}{}$	3	65.44	26.44	8.12

* Some enamel missing.

† Some of the sample lost during separation of dentin and enamel.

Part b. Summary of Data

	ALL THE TEETH			THE NON-CARIOUS TEETH			THE CARIOUS TEETH		
	Max.	Min.	Aver.‡	Max.	Min.	Aver.	Max.	Min.	Aver.
Dentin....	72.31	39.42	60.51	72.31	39.42	60.15	69.76	46.45	62.48
Enamel....	42.96	8.79	27.83	41.03	12.14	28.24	42.96	22.10	25.78
Loss.....	25.40	7.34	11.70	25.40	7.34	11.21	21.45	6.91	11.74

‡ Teeth Nos. 28, 22, 14 not included.

per cent, respectively) do not differ significantly from those from the non-carious teeth (60.2 and 28.2 per cent). These figures are somewhat different from similar data on modern teeth (2) in which the proportion of enamel ranges from 30 to 40 per cent of the entire tooth.

The lower values reflect the effects of attrition but may also be due in part to a greater loss of the enamel in the separation process. The percentage loss in most cases seems high. This may be accounted for in part by the fact that the very dry and often chalky teeth dusted badly when powdered.

The analytical data for the dentin and the enamel of 30 teeth are given in Table III. Specifically the percentage of inorganic material in all the dentin and all the enamel, and the percentages of Ca, P, CO_2 and the Ca:P ratio of the inorganic portions of each are listed. Table III, part a, shows also the tooth position and caries "class."

Table III, part b (1) gives the over-all ranges, the averages and the standard deviations of percentages of inorganic matter, Ca, P, CO_2 and Ca:P ratios for all the teeth and for the sound and carious teeth separately.

The few available data on fossil teeth (with the exception of Greene's analyses, Table V) are given in Table IV. The principal interest of these analyses is the constancy in tooth composition regardless of analyst, geological period or species of animal. The Ca:P ratios are frequently higher than those for modern animal teeth which may indicate an increase in the calcium perhaps as a result of exposure to calcium-rich water.

Enamel: In Table V are given the analyses of modern, precolumbian and fossil enamel and dentin. The enamel values are amazingly similar for each constituent excepting the CO_2 of which the precolumbian teeth have about 30 per cent less than the modern teeth and 40 per cent less than the fossil teeth. The enamel phosphorus values are fairly constant; the calcium values are highest for the precolumbian teeth, consequently the Ca:P ratios are higher for precolumbian than for modern teeth. It is of interest to note that the Ca:P ratio in each case is close to 2.15, the theoretical value for hydroxylapatite.

Dentin: The dentin calcium and phosphorus values are higher for precolumbian than for modern teeth, but the CO_2 values are 10 per cent lower (compare enamel values). The fossil dentin values are much higher for each constituent than in the recent teeth, in fact, they approach the values for enamel. Hypothetically, this increase from modern to precolumbian to fossil might be described as a mineralization of the dentin in which the organic structures are gradually

TABLE III

Part a. Analysis of Dentin and Enamel (Dry Weight)

TOOTH			DENTIN PER CENT					ENAMEL PER CENT				
No.	Position	Caries class	Inorganic	Ca	P	Ca/P	CO_2	Inorganic	Ca	P	Ca/P	CO_2
6	$\frac{1}{1}$	0	81.24	31.80	13.74	2.31	3.98	98.73	35.19	17.00	2.07	1.52
8	$\frac{1}{4}$	0	79.32	35.47	16.81	2.11	3.07	98.80	37.35	17.86	2.09	1.89
32	$\frac{1}{4}$	0	79.00	36.78	16.45	2.24	3.12		37.28	17.53	2.13	2.03
21	$\frac{1}{7}$	0	75.72	36.76	15.81	2.33	4.09	97.26	37.28	17.08	2.18	1.85
27	$\frac{1}{8}$	1	83.80	37.17	17.09	2.18	3.94					
15	$\frac{1}{1}$	0	78.47	35.67	15.92	2.24	2.37	97.88	37.42	17.85	2.10	1.98
28	$\frac{1}{1}$	0						98.54	38.30	18.60	2.46	
30	$\frac{3}{1}$	0	75.26	37.23	17.48	2.13	2.67	95.48	37.82	18.03	2.10	1.55
31	$\frac{4}{1}$	0	78.46	37.77°	18.28	2.07	2.40	98.41	37.45	17.61	2.13	1.93
19	$\frac{6}{1}$	0	80.70	35.69	16.22	2.20	3.14					
20	$\frac{6}{1}$	0	64.50*	36.72	15.67	2.34	3.08†	*	35.27	16.70	2.11	1.72
7	$\frac{7}{1}$	0	80.53	34.82	16.99	2.07	4.01	99.04	37.26	18.61	2.00	2.50
18	$\frac{7}{1}$	0	77.20	37.31	17.37	2.15	3.86	98.56	38.62	17.80	2.17	2.59
29	$\frac{8}{1}$	1										
2	$\frac{1}{1}$	0	82.19	31.59	14.10	2.24	2.63	97.70	37.72	18.10	2.08	2.14
9	$\frac{1}{1}$	0	81.43	35.10	16.80	2.07	2.09	98.70	37.43	18.01	2.08	2.95
10	$\frac{2}{1}$	0	81.39	35.22	16.90	2.08	3.28	98.80	36.89°	17.99	2.05	1.89
1	$\frac{3}{1}$	0	77.73	36.07	17.25	2.09	3.27	96.18	35.47	17.45	2.03	1.71
24	$\frac{5}{1}$	0	84.54	37.24	17.02	2.19	2.79	96.27	37.11	17.79	2.09	1.85
13	$\frac{6}{1}$	1	80.86	36.27	16.84	2.15	3.59	98.20	37.60	17.55	2.14	1.31
16	$\frac{6}{1}$	0	79.15	34.60°	17.14	2.27	3.50	99.00	37.93	17.26	2.20	2.48
22	$\frac{6}{1}$	0						98.31	36.80	17.05	2.16	1.85
4	$\frac{7}{1}$	0	80.01	34.72	17.32	1.99	3.37	98.00	38.04	17.82	2.14	2.68
3	$\frac{8}{1}$	0	81.44	35.61	16.79	2.12	4.39	*	35.09	16.31	2.15	1.67
5	$\frac{8}{1}$	0	62.15*	34.77	16.74	1.95	3.04	99.25	36.52	17.87	2.04	2.53
11	$\frac{2}{1}$	0	80.34	35.61	16.78	2.12	3.66	97.00	34.92°	16.12	2.17	2.13
17	$\frac{2}{1}$	0	78.03	37.32°	17.67	2.11	3.36	98.90	38.19	17.65	2.16	1.88
14	$\frac{3}{1}$	0	69.26*	32.95	15.28	2.16	3.70	99.60	36.79°	17.84	2.06	2.19
26	$\frac{3}{1}$	0	79.90	36.81	17.22	2.14	3.19	94.40	38.08	18.01	2.11	2.51
23	$\frac{5}{1}$	0	76.63	35.30°	16.75	2.11	3.66	98.50	37.15	17.96	2.07	1.73
12	$\frac{6}{1}$	1	82.60	35.61	16.59	2.15	3.91	98.30	38.26	17.91	2.14	2.03
25	$\frac{6}{1}$	3	83.00	37.37	17.33	2.15	2.82	96.55	37.42	17.63	2.12	2.08
No. of teeth....			29	29	29	29	29	27	30	30	30	29
Average.....			79.95	35.70	16.63	2.15	3.31	97.97	37.13	17.62	2.12	2.05

° Single determination.

† Duplicate determination.

* Some lost in oven; not included in averages.

lost or replaced by calcium phosphates. This process may involve filling of the tubules with calcific materials. The Ca:P ratio increases in the same way, the value for fossil dentin indicating a higher calcium content than is required for hydroxyl apatite, and reflecting the disproportionately greater Ca content of the fossil teeth. The Ca:P ratio of precolumbian teeth is exactly that of hydroxyl apatite. The stability of the tooth calcium phosphates subjected to unknown but presumably various conditions of soil water over a long period of time

TABLE III

Part b. 1. Dentin of Precolumbian Teeth

TOTAL					SOUND				CARIOUS			
No. Teeth	Constituents	Per cent Composition			No. Teeth	Per cent Composition			No. Teeth	Per cent Composition		
		Maximum	Average ± stand. dev.	Minimum		Maximum	Average ± stand. dev.	Minimum		Maximum	Average ± stand. dev.	Minimum
29	Ca	37.77	35.70 ±1.31	31.59	25	37.77	35.58 ±1.40	31.59	4	37.37	36.60 ±0.71	35.61
29	P	17.67	16.63 ±0.94	13.74	25	17.67	16.58 ±0.94	13.74	4	17.33	16.96 ±0.28	16.59
29	Ca/P	2.34	2.15 ±0.09	1.95	25	2.34	2.16 ±0.09	1.95	4	2.18	2.16 ±0.02	2.16
29	CO_2	4.39	3.31 ±0.56	2.09	25	4.39	3.27 ±0.56	2.09	4	3.94	3.57 ±0.45	3.57
26	Inorganic	84.54	79.95 ±2.34	75.26	22	84.54	79.49 ±2.12	75.26	4	83.80	82.56 ±1.08	82.56

2. Enamel of Precolumbian Teeth

TOTAL					SOUND				CARIOUS			
No. Teeth	Constituents	Per cent Composition			No. Teeth	Per cent Composition			No. Teeth	Per cent Composition		
		Maximum	Average stand. dev.	Minimum		Maximum	Average stand. dev.	Minimum		Maximum	Average stand. dev.	Minimum
30	Ca	38.62	37.13 ±0.99	34.92	26	38.62	37.05 ±0.99	34.92	4	38.26	37.65 ±0.37	37.31
30	P	18.61	17.62 ±0.49	16.00	26	18.61	17.61 ±0.49	16.00	4	17.91	17.54 ±0.18	17.55
30	Ca/P	2.46	2.12 ±0.05	2.00	26	2.46	2.12 ±0.05	2.00	4	2.14	2.13 ±0.01	2.12
30	CO_2	2.95	1.98 ±0.39	1.13	26	2.95	1.99 ±0.39	1.13	4	2.26	1.92 ±0.55	1.31
27	Inorganic	99.60	97.97 ±1.24	94.40	23	99.60	98.39 ±1.35	94.40	4	98.91	97.99 ±0.88	96.55

is especially noteworthy. This stability is a typical property of hydroxyl apatite in weakly acid, neutral or alkaline solutions.

Carbonate: The lower CO_2 values in enamel of precolumbian teeth as compared to modern or fossil teeth is puzzling. The preferential removal of carbonates by weak acids, such as soil water may contain, could explain the decrease from modern to precolumbian tooth values. Why fossil enamel and dentin should have higher CO_2 values than modern precolumbian is not known. However, the deposition of

calcium carbonate in the mineralization of the organic material would account for the increase in CO_2 content and also for part of the higher Ca values.

TABLE IV

Analyses of Fossil Teeth

ANALYST	HOPPE (8)				GROSSMAN (9)	REYNOLDS ET AL. (10)		BRUBAKER* (11)			
Tissue	Enamel				Whole tooth[5]	Whole tooth		Dentin[7]			
	VI[1]	VII[2]	VIII[3]	IX[4]		Fish	Animals[6]				
Ca	37.55	37.82	38.21	37.90	39.90			38.2	39.1	38.6	36.8
P	18.08	18.08	17.52	17.84	16.95	16.22	16.98	16.1	16.1	16.65	15.72
CO_2	5.88	6.36	8.63	7.02	4.02			7.92	6.62	6.83	6.83
Ca/P	2.06	2.09	2.18	2.11	2.35			2.37	2.43	2.32	2.34
H_2O					8.20			3.16	3.38	3.92	5.53
Org.	3.16	4.54	1.24	2.32	25.20			3.38	4.22	5.42	19.00
Inorg.	96.84	95.46	98.76	97.68	66.60			93.46	92.40	90.66	75.47

* Analyses by Moling.

[1] Fossil Rhinocerus molar Bessarabia.

[2] Fossil Elephant molar Bessarabia.

[3] Fossil Mastodon Alps.

[4] Paleotherium Alps.

[5] 200–50 B.C. Human.

[6] Land pebble phosphate of Florida.

[7] Tusks of extinct Elephants, Kansas.

TABLE V

Comparison of Analyses of Modern, Precolumbian and Fossil Enamel and Dentin
(Dry wt. basis)

	ENAMEL			DENTIN		
	Modern (12)	Precolumbian	Fossil Titanotherium (13)	Modern (13)	Precolumbian	Fossil-Titanotherium (13)
Ca	36.10	37.7	37.0	26.1	28.4	35.6
P	17.3	17.9	17.2	12.6	13.2	15.8
CO_2	3.05	2.02	3.57	2.94	2.63	3.20
Ca/P	2.09	2.12	2.16	2.07	2.15	2.26

Comparing the average values for the various constituents of the mineral phase of precolumbian with those of modern dentin, none has a statistically significant difference.

Constancy: The constancy of composition of modern, precolumbian and fossil teeth is more clearly shown by expressing the constituents as moles compared to Ca set at 10 (Table VI). The reason for choosing this system is that in hydroxyl apatite the moles of Ca:P:OH are 10:6.0:2.0. In each case, both in enamel and in dentin, the Ca and P mole values are very close to the 10:6 ratio with the exception of fossil dentin already discussed. From modern to precolumbian to fossil teeth, the CO_2 content in enamel varies irregularly but in dentin there is a progressive decrease. The CO_2 moles are near 1, which is the basis for the hypothesis of a carbonate apatite existing in teeth (carbonate apatite = Ca—10:P—6.0:CO_2—1.0) suggested by earlier writers. However, the presence of appreciable amounts of a carbonate apatite are ruled out on crystallographic grounds (14, 15).

TABLE VI

Constancy of Composition of Modern, Precolumbian and Fossil Enamel and Dentin Expressed in Moles relative to Ca set at 10

	ENAMEL			DENTIN		
	Modern	Pre-columbian	Fossil	Modern	Pre-columbian	Fossil
Ca	10	10	10	10	10	10
P	6.2	6.1	6.0	6.2	6.0	5.7
CO_2	0.77	0.49	0.88	1.02	0.84	0.82

SUMMARY

1. The analysis of 32 precolumbian teeth are reported. Five of these teeth are carious, 26 showed attrition and 9 gave evidence of periodontal resorption and calculus.

2. The enamel and dentin were separated. About 30 per cent enamel, 60 per cent dentin, and 10 per cent loss were obtained.

3. The percentage composition of the enamel inorganic material averaged Ca—37.1, P—17.6, CO_2—1.98, Ca:P—2.12 (on the dry weight basis, inorganic—98.0 per cent).

4. The percentage composition of the dentin inorganic material averaged Ca—35.7, P—16.6, CO_2—3.31, Ca:P—2.15 (on the dry weight basis, inorganic—80.0 per cent).

5. No significant differences between averages for sound and carious teeth were found.

6. The constancy of composition of precolumbian teeth is noteworthy. The mole compositions of the mineral phase of precolumbian enamel and dentine are nearly the same as those of modern and fossil teeth.

7. The calcium and phosphorus values are near the theoretical for hydroxylapatite.

The authors acknowledge the assistance of Dr. Aleš Hrdlička, of Dr. Marian L. Manly and of Dr. Virgil D. Cheyne.

REFERENCES TO LITERATURE

1. Manly, R. S., and Hodge, H. C., *J. D. Res.*, **18:** 133, 1939.
2. LeFevre, M. L., and Manly, R. S., *J. A. D. A.*, **25:** 233, 1938.
3. French, E., Welch, E. A., Simmons, J., LeFevre, M. L., and Hodge, H. C., *J. D. Res.*, **17:** 401, 1938.
4. Clarle, E. P., and Collip, J. B., *J. Biol. Chem.*, **63:** 461, 1925.
5. LeFevre, M. L., and Nicholson, E., *J. D. Res.*, **17:** 31, 1938.
6. Holtz, F., *Biochem. Z.*, **210:** 252, 1929.
7. Shear, M. J., and Kramer, B., *J. Biol. Chem.*, **79:** 105, 1928.
8. Hoppe, F., *Arch. path. Anat. u. Physiol.*, **24:** 13, 1862.
9. Gassmann, T., *Z. physiol. Chem.*, **63:** 397, 1909.
10. Reynolds, D. S., Jacobs, K. D., and Hill, W. L., *Ind. Eng. Chem.*, **21:** 1253, 1929.
11. Brubaker, H. W., *Trans. Kans. Acad. Sci.*, **37:** 115, 1934.
12. Armstrong, W. D., and Brekhus, P. J., *J. Biol. Chem.*, **120:** 677, 1937.
13. Greene, F. V., *Amer. Jour. Sci. Arts*, (Silliman and Dana) Series II, **16:** 16, 1853.
14. Bale, W. F., *Zeit. Kristal.*, in press.
15. Thewlis, J., Glock, G. E., and Murray, M. M., *Trans. Farad. Soc.*, **35:** 358, 1939.

A METHOD FOR MICRODETERMINATION OF DENSITY IN CALCIFIED TISSUES[1]

MARTIN DEAKINS, D.D.S., M.S. AND RICHARD S. MANLY, PH.D.

From the Department of Biochemistry and Pharmacology, School of Medicine and Dentistry, The University of Rochester, Rochester, New York

To determine the density of micro-quantities of calcified tissues, a method has been devised for the direct volume measurement of blocks of the tissue cut out with a micro-jigsaw. This avoids contamination with organic liquids employed in the usual flotation micro-procedures. Subsequent handling and transfer of the blocks are easier than in the case of powdered samples. Since only 1 to 4 cu. mm. of material are necessary for a determination, various zones of calcified tissues of small animals may be investigated. Furthermore, a direct volume determination was obtained, a fact of greater importance in our investigation of carious dentin. This made possible a measurement of void volume, and the estimation of the absolute changes in ash, organic, and water contents which occur in carious zones of human dentin.

DESCRIPTION OF APPARATUS

The upper end of a jeweler's saw blade is attached to the needle carrier of a sewing machine. The lower end of the blade is clamped with a set-screw to a ¼ inch piston sliding vertically along the same axis. A coiled spring at the lower end of the piston produces the tension and downstroke of the blade. The saw table or stage is a wide microscopic slide with a 0.5 mm. hole through the center, through which the blade travels. Trans-illumination is obtained with a ribbon filament lamp and mirror.

PROCEDURE

Longitudinal sections are cut from teeth with a circular saw and ground plano-parallel to about 0.50 ± 0.01 mm. Irregular carious and normal areas, weighing 0.5 to 10 mg., are sawed from the section with the micro-jigsaw shown in *fig. 1*. The section is held on the saw table with probes while being cut, and is observed through a 10× binocular micro-

[1] Presented to the Microchemical Division of the American Chemical Society at Baltimore, Md., April 4, 1939.

scope. A drop of water is added occasionally to prevent heating and loss of moisture. After some practice, one can obtain blocks with sides perpendicular to the ground surfaces and with a diameter less than 1 mm. The thickness and greatest diameter of the block are measured with a ball point micrometer and an ocular micrometer, respectively. The area is determined by planimetry from a 30× photographic enlargement (1).

FIG. 1

From these values the volume is expressed by

$$\left[\frac{B}{P \times O}\right]^2 \times A \times T \times 645 = \text{volume (cu. mm.)}$$

where O = constant for ocular micrometer (divisions per mm.)
B = greatest diameter of block (divisions)
P = greatest diameter of photograph of block (mm.)
A = area of photograph in sq. in.
T = thickness of block in mm.
645 = conversion factor, sq. in. to sq. mm.

The weight, determined on a micro-balance, divided by this volume is equal to the density of the block.

The precision of the volume determination was obtained from 10 measurements on each of 2 blocks of normal dentin, one of minimum and the other of medium size. The probable error of a single deter-

mination amounts to 1.3 per cent for the smaller (1.01 cu. mm.) and 0.9 per cent for the larger (4.35 cu. mm.)

TABLE I

Precision of the volume determination

DETERMINATION NUMBER	BLOCK #1 VOL., MM.^{3}	BLOCK #2 VOL., MM.^{3}
1	0.981	4.281
2	1.031	4.420
3	1.019	4.424
4	1.033	4.355
5	1.018	4.383
6	1.036	4.357
7	1.023	4.382
8	1.024	4.349
9	0.986	4.217
10	1.002	4.314
Average	1.0153	4.3482
Probable error of a single determination:		
mm.^{3}	0.013	0.035
per cent	1.3	0.8

TABLE II

Density of various types of dentin

CLASSIFICATION	NO. OF ZONES STUDIED	AVERAGE DENSITY
Normal crown	26	2.112
Normal root	14	2.061
Secondary dentin	3	1.97
Transparent, near caries	6	1.958
Cementum	3	1.739
Hard, pigmented caries	5	1.90
Soft, pigmented caries	3	1.44

Average values for the density of various types of dentin are given in Table II. The density of root dentin is significantly lower than that of normal crown dentin (2.5 per cent); these values agree well with the findings of Black (2). Both forms of translucent dentin—"secondary," formed in the pulp cavity, and transparent near caries—are

about 5 per cent less dense than the crown. Next in order of decreasing density come the hard (undecalcified), pigmented areas of caries and cementum (up to 10 per cent less than the crown.) A precipitous drop in density is occasioned by the loss of mineral salts from soft, pigmented dentin, where the density is diminished on the average by 40 per cent.

SUMMARY

A micro-method has been devised for determining the density (or porosity) of calcified tissues by directly measuring the volume of blocks prepared by a micro-jigsaw. The relative precision is 1.3 per cent for 1 cu. mm. samples. A table of average values for the densities of various normal and carious zones of human dentin is included.

REFERENCES TO LITERATURE

1. Boyd, J. D., Drain, C. L., and Deakins, M., *J. D. Res.* **17**: 465, 1938.
2. Black, G. V., *D. Cosmos* **37**: 353, 1895.

MEASUREMENTS OF THE ASCENDING RAMUS OF THE MANDIBLE

BÉLA SZOKOLÓCZY-SYLLABA, Dr. Med.

Stomatological Clinic, Royal Hungarian University, "Pázmány Péter", Budapest, Hungary

A technique for intraoral nerve block anesthesia, in which the position of the lingula mandibularis is determined, has been described.[1] The angle of the mandible and the posterior border of the ramus are considered in addition to the usual intraoral structures and by the aid of these landmarks, and without regarding of the position of the teeth, the lingula can be located.

The technique is described for the use of the left hand on either side of the patient. For block anesthesia of the right inferior alveolar nerve the third finger of the left hand is placed on the posterior border of the mandible. The fourth finger is placed below so that it rests on the inferior posterior portion of the mandible near the angle. The second finger is placed close to the third outside the mouth. The first finger is now placed inside the mouth at the level of the second finger in the retromolar triangle. Midway between the first and second fingers is the antero-posterior position of the lingula (*fig. 1*). On the left side the technique is similar except that the hand is turned over so that the positions of the second, third and fourth fingers are inverted. Thus, on the left side the lingula's position will be midway between the first finger (in the mouth) and the fourth finger (on the posterior edge of the mandible) (*fig. 2*).

This technique has been employed in our clinic since 1933, during which time not only antero-posterior variations in the position of the lingula have been observed, but also variations in the lateral angle between the ramus and body of the mandible. It has been observed that the lateral position of the ramus is more definitely related to the supporting structures on the lingual side of the molars and premolars,

[1] Szokolóczy Syllaba, B.: *Memorial Book of the Stomatological Clinic*, (Budapest, 1933), p. 194.

especially to the molars with their marked lingual inclinations. The lateral deflection of the anterior border of the ramus and the lingual inclination of the alveolar border of the body of the mandible in the molar region must be considered by the anesthetist to correctly direct his needle. The following general rules for double angulation have been used: (a) Where the lateral inclination of the anterior border of the ramus is great the syringe is placed more toward the opposite teeth. (b) Where the ascending ramus is broad the needle is placed more deeply into the soft tissue.

FIG. 1

FIG. 2

Skeletal material was used to verify the observations made clinically. A modified goniometer was constructed, consisting essentially of 2 right angles or L's rotating on a common axis with scales for reading the angle between and also the distances along one arm (*fig. 3*). The ungraduated arm of the larger L is placed on the lingual supporting structures of the last 2 molars at the alveolar border. The other L is moved along until it lies immediately over the lingula on the inner surface of the ramus. The smaller L is rotated to record the deflection of the ramus from the body of the mandible (*fig. 4*).

In this way measurements were made on 105 mandibles to ascertain (a) the angle of deflection of the ramus from the body, (b) the dis-

tances between the 2 temporal crests and the 2 posterior margins at the level of the lingula, (c) the breadth of the ascending ramus from the crista temporalis to the posterior margin and from the

FIG. 3

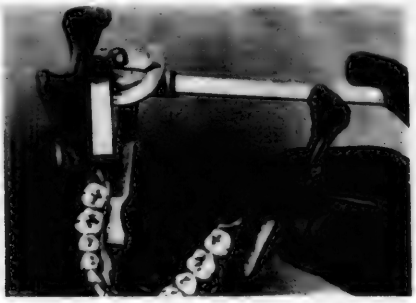


FIG. 4

TABLE I

Deflection of the ascending ramus (angle between ramus and body of mandible)

Deflection (degrees)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
No. of cases	4	1	5	9	7	6	9	12	15	12	13	1	5	1	0	1	4

TABLE II

Distance between crista temporalis and posterior border of ramus (breadth)

Breadth of ramus (mm.)	22	23	24	25	26	27	28	29	30	31	32	33
No. of cases	1	3	13	17	16	15	11	17	6	3	3	1

TABLE III

Distance between foramen mandibularis and inferior border of mandible

Distance (mm.)	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
No. of cases	1	4	2	4	4	10	14	9	14	4	7	7	7	4	6	2	2	3	1	1	1

anterior to the posterior margin at the level of the lingula and, (d) the distance from the inferior border to the foramen mandibularis. The results of these measurements are given in Tables I, II, and III.[2]

[2] Complete records of the individual measurements have been made but are omitted here to conserve space.

Results of these measurements may be summarized as follows: (1) The deflection of the ramus varied widely from 2 to 18 degrees with a median of 10 degrees. (2) The breadth of the ramus from the crista temporalis (torus verticalis of Lenhossek) to the posterior border at the level of the lingula was from 22 to 33 mm. with median values from 24 to 29 mm. (3) The distance from the inferior border of the mandible to the foramen mandibularis varied between 17 and 37 mm. with median values of 22 to 25 mm.

Data for the distances between the posterior margins and the temporal crests is not given in the tables. These values give the deflection of the ramus strictly in relation to the median line. By the sine theory the values were calculated into degrees and variations from the values found with the goniometer were noted. However, the anesthetist is more interested in the angle between the ramus and the body than that between the ramus and median plane.

The following conclusions may be of value in administering mandibular block anesthesia: (1) Variations of the mandible are such as to warrant consideration in the administration of local anesthesis. (2) The intraoral structures, alone, do not suggest the extreme variations.

By the use of the method described the differences in individuals' measurements can be approximated because the fingers on the external surface indicate the median value of the position of the foramen mandibularis and the fingers also indicate the breadth and angle of deflection of the ramus.

SUPPLEMENT

CONTENTS

I. Sustaining subscribers to the *Journal of Dental Research*, 1939. Compiled by M. W. McCrea, Circulation Manager 565
II. International Association of Dental Research. Register of members by sections, as of December 10, 1939. Compiled by the General Secretary 566
III. Proposed new plan of dental education at Harvard. L. M. S. Miner 570

I. SUSTAINING SUBSCRIBERS TO THE JOURNAL OF DENTAL RESEARCH, 1939[1]

Compiled by M. W. McCrea, Circulation Manager

DR. GEORGE M. ANDERSON, *Baltimore.*
DR. J. L. T. APPLETON, *Philadelphia.*
DR. M. S. AISENBERG, *Baltimore.*
DR. R. E. BLACKWELL, *Chicago.*
DR. O. W. BRANDHORST, *St. Louis.*
DR. O. J. CHASE, JR., *New York.*
DR. A. E. CORBY, *New York.*
DR. C. G. DARLINGTON, *New York.*
DR. B. E. ERICKSON, *Washington.*
DR. L. E. FORD, *Los Angeles.*
FIRST DISTRICT DENTAL SOCIETY, *New York.*
DR. L. I. GROSSMAN, *Philadelphia.*
DR. J. O. GOODSELL, *Saginaw.*
DR. E. H. HATTON, *Chicago.*
DR. H. L. HANSEN, *Chicago.*
DR. I. HIRSCHFELD, *New York.*
DR. S. HEMLEY, *New York.*
DR. H. W. KROGH, *Washington.*
DR. R. KRONFELD, *Chicago.*
DR. P. C. KITCHEN, *Columbus*
DR. M. KARSHAN, *New York.*
DR. A. L. KOHN, *New York.*
DR. F. KRASNOW, *New York.*
DR. H. J. LEONARD, *New York.*
DR. W. H. G. LOGAN, *Chicago.*
DR. H. LYONS, *Richmond.*
DR. J. O. MCCALL, *New York.*
DR. J. NASSAU, *Hartford.*
DEPT. OF PATHOLOGY, NEW YORK UNIVERSITY, DENTAL SCHOOL.
DR. J. B. ROBINSON, *Baltimore.*
DR. R. W. RULE, *San Francisco.*
DR. R. J. RINEHART, *Kansas City.*
DR. H. M. SEMANS, *Columbus.*
DR. W. SOUDER, *Washington.*
DR. I. SCHOUR, *Chicago.*
DR. W. G. SKILLEN, *Chicago.*
DR. G. R. SPALDING, *Birmingham.*
DR. W. W. TRACY, *New York.*

---

[1] Sustaining subscribers have materially aided the publication of this volume by payment of $10 or more. These monies are used for current expenses.

II. International Association for Dental Research[1]

Register of Members by Sections[2, 3]

Compiled by the General Secretary

1. *Ann Arbor Section.*—R. K. Brown, R. W. Bunting, Mary C. Crowley, M. C. Dixon, K. R. Easlik, J. O. Goodsell, **Philip Jay*, R. H. Kingery, G. R. Moore, F. A. Peyton, E. O. Scott, F. B. Vedder, M. L. Ward.—13.

2. *Baltimore Section.*—**M. S. Aisenberg*, G. M. Anderson, E. C. Dobbs, Harold Goldstein, H. E. Kelsey, E. V. McCollum, M. W. McCrea, J. Ben Robinson, A. H. Schultz.—9.

3. *Boston Section.*—L. W. Baker, D. D. Bloom, F. R. Blumenthal, P. E. Boyle, W. G. Bridge, F. R. Cushman, F. H. Daley, H. M. Goldman, C. E. Hatch, J. C. Healy, E. A. Hooton, P. R. Howe, V. H. Kazanjian, P. A. Leavitt, Howard Majerison, F. P. McCarthy, L. M. S. Miner, A. L. Morse, **Fred W. Morse, Jr.*, R. E. Nagle, S. E. Pond, A. P. Rogers, Benjamin Spector, K. H. Thoma, Harry Trimble, H. L. Weatherford, David Weisberger, G. H. Wright.—28.

4. *Budapest Section.*—Károly Balogh, István Bánhegyi, Béla Bonyhárd, János Brand, Béla de Simon, Károly Ferenczy, Elek Földi, Dezsö Hattysazy, Oszkár Kömives, **Ferenc Köszeg*, Ervin Landgraf, Gusztáv Morelli, Rudolph Rehák, Henrik Salamon, László Sugár, Steven Varga, Dénes von Máthé.—17.

5. *Chengtu Section.*—Mary C. Agnew (Mrs. R. G.), **R. G. Agnew*, H. B. Collier, L. G. Kilborn, A. W. Lindsay, H. J. Mullet.—6.

6. *Chicago Section.*—H. H. Ascher, Olaf Bergeim, J. R. Blayney, E. P.

[1] The proceedings of the *seventeenth* general meeting (Cleveland, March 18–19, 1939) were published in the *J. D. Res.*, 18, 213, 1939 (this volume).

[2] *Sectional meetings in 1938–1939* were held on the following schedule as reported by sectional secretaries. *Ann Arbor*—May. *Boston*—Jan. 9, Feb. 6, Mar. 6, May 8. *Chicago*—Oct. 20, Jan. 20, April 21, May 19. *Iowa City*—Jan. 10. *New York*—Jan. 19, June 1. *Philadelphia*—Oct. 29, Dec. 16. *Richmond*—Jan. 27. *St. Louis*—Nov. 29, Mar. 26, Dec. 10, Mar. 27, Dec.—. *Washington*—Nov. 29, Mar. 19, April 11, Dec.—

[3] The following members represented their sections in Council during 1939 (named in alphebetical order of sections): P. Jay, M. S. Aisenberg, L. M. S. Miner, (Budapest-none), R. G. Agnew, I. Schour, T. J. Hill, P. C. Kitchin, P. C. Lowery, J. S. Bagnall, E. Kotanyi, A. W. Bryan, G. Friel, E. Sprawson, G. VanHuysen, P. J. Brekhus, (New Haven-none), S. L. Tiblier, L. M. Waugh, T. J. Cook, H. E. Friesell, F. Neumann, H. Bear, J. Volker, L. R. Main, W. C. Fleming, A. J. McDonagh, R. Trauner, L. G. Jordan (11/21/39—H. T. Dean), H. J. Merkeley. In addition W. S. Crowell represented the Materials Group.

* Section Secretary.

Boulger, S. F. Bradel, A. G. Brodie, R. S. Claflin, S. W. Clark, E. D. Coolidge, A. A. Dahlberg, G. B. Denton, W. A. Figg, L. S. Fosdick, C. W. Freeman, S. M. Gordon, Carolyn Hammond, H. L. Hansen, R. W. Harrison, E. H. Hatton, M. K. Hine, M. M. Hoffman, Oscar Kanner, F. F. Kanthak, W. M. Kern, R. G. Kesel, Rudolph Kronfeld, R. A. Lasater, A. B. Luckhardt, W. H. G. Logan, G. R. Lundquist, B. F. Miller, A. H. Mueller, Emil Mueller, F. B. Noyes, H. J. Noyes, V. T. Nylander, **E. C. Pendleton*, Balint Orban, H. A. Potts, Isaac Schour, W. R. Schram, W. G. Skillen, E. W. Skinner, R. M. Stephan, N. G. Thomas, Ruth Tunnicliff, S. D. Tylman, E. C. Wach, Friederich Wasserman, W. H. Welker, R. C. Willett, Warren Willman, Joseph Weinmann, H. A. Zander, E. A. Zaus.—55.

7. *Cleveland Section.*—B. H. Broadbent, **S. W. Chase*, R. P. Dressel, T. J. Hill, W. M. Krogman, V. C. Myers, W. A. Price, C. H. Scheu, P. B. Taylor, C. J. Vosmik, F. C. Waite.—11.

8. *Columbus Section.*—C. O. Boucher, H. C. Brown, J. B. Brown, A. J. Bush, H. V. Cottrell, L. F. Edwards, W. C. Graham, J. H. Kaiser, **P. C. Kitchin*, H. W. McMillan, R. D. McFarland, L. S. Pettit, S. J. Randall, H. M. Seamans.—14.

9. *Detroit Section.*—**S. J. Lewis*, P. C. Lowery.—2.

10. *Halifax Section.*—J. S. Bagnall, R. J. Bean, H. M. Eaton, A. W. Faulkner, A. B. Haverstack, Donald Mainland, W. C. Oxner, **S. G. Ritchie*, E. G. Young.—9.

11. *Houston Section.*—F. C. Elliott, **E. Kotanyi*, W. H. Scherer.—3.

12. *Iowa City Section.*—A. W. Bryan, G. S. Easton, L. B. Higley, **A. O. Klaffenbach*, T. D. Speidel, R. H. Volland.—6.

13. *Johannesburg Section.*—G. Friel, J. N. Noriskin, J. C. M. Shaw, Julius Staz, **W. A. Wilson.*—5.

14. *London Section.*—Arthur Bulleid, J. F. Colyer, E. B. Dowsett, E. W. Fish, C. W. Howkins, G. B. Pritchard, **Evelyn Sprawson*, Joseph Turner.—8.

15. *Louisville Section.*—Brooks Juett, H. T. Knighton, **R. E. Myers*, J. T. O'Rourke, K. R. Pfeiffer, W. M. Randall, Grant Van Huysen.—7

16. *Minnesota Section.*—W. D. Armstrong, E. S. Best, P. J. Brekhus, J. T. Cohen, I. A. Epstein, B. S. Gardner, C. J. Grove, W. F. Lasby, J. F. McClendon, F. S. Meyer, C. E. Rudolph, Dorothy F. Radusch, W. J. Simon, E. S. Stafne, N. O. Taylor, W. D. Vehe, H. F. Wahlquist, C. F. Waldron, **H. G. Worman.*—19.

17. *New Haven Section.*—**B. G. Anderson*, C. G. Burn, J. J. Wolfe.—3.

18. *New Orleans Section.*—S. L. Tiblier, **C. S. Tuller*, F. J. Wolfe.—3.

19. *New York Section.*—Edmund Applebaum, H. A. Bartels, Adolph

* Section Secretary.

Berger, F. E. Beube, Gerrit Bevelander, Theodor Blum, C. F. Bodecker, W. A. Bossert, R. H. Brodsky, L. R. Cahn, M. W. Carr, G. W. Clapp, W. H. Crawford, C. G. Darlington, Gershom Downs, H. S. Dunning, W. B. Dunning, H. S. Dwyer, Genevieve Foley, Raymond Gettinger, W. J. Gies, W. K. Gregory, Milo Hellman, Samuel Greenburg, Samuel Hemley, I. Hirschfeld, Houghton Holliday, Franklin Hollander, R. G. Hutchinson, Jr., T. P. Hyatt, A. L. Johnson, Theodore Kaletsky, Maxwell Karshan, T. B. King, Frances Krasnow, William Lefkowitz, H. J. Leonard, E. C. McBeath, J. O. McCall, Frederick McKay, A. H. Merritt, E. G. Miller, Jr., S. C. Miller, Egon Neustadt, **Isaac Neuwirth*, A. T. Newman, Edith Oblatt, B. B. Palmer, Theodor Rosebury, W. M. Rogers, Irving Salman, J. A. Salzman, M. I. Schamberg, Joseph Schroff, H. H. Shapiro, E. H. Siegel, H. F. Silvers, Sidney Sorrin, F. L. Stanton, George Stein, M. R. Stein, P. R. Stillman, David Tanchester, Benjamin Tenenbaum, L. M. Waugh, J. L. Zemsky, D. E. Ziskin.—67.

20. *Philadelphia Section.*—J. L. T. Appleton, L. W. Burkett, Carrie K. Bryant, H. R. Churchill, **T. J. Cook*, W. S. Crowell, L. I. Grossman, R. H. Ivy, J. V. Mershon, H. A. Miller, Herman Prinz, C. R. Turner.—12.

21. *Pittsburg Section.*—W. H. Archer, T. W. Brand, G. J. Cox, J. J. Enright, W. L. Fickes, F. C. Friesell, H. E. Friesell, T. F. McBride, E. G. Meisel, J. S. Oartel, N. C. Ochsenhirt, W. F. Swanson, **L. E. Van Kirk*, E. A. Wolfe, W. H. Wright.—15.

22. *Prague Section.*—Karel Černý, Karl Häupl, Ján Jesenský, František Kostečka, Jaromir Křečan, A. E. Loos, F. Náprstek, **Friedrich Neumann*, František Neuwirth, Čestimir Parma, Josef Přibyl, Ferdinand Škaloud, Karel Wachsmann, Hans Wermuth.—14.

23. *Richmond Section.*—S. S. Arnim, Harry Bear, A. D. Brashear, O. W. Clough, H. D. Coy, J. C. Forbes, **J. F. Hall*, A. P. Little, Harry Lyons, J. B. Williams.—10.

24. *Rochester Section.*—Frank Allen, B. G. Bibby, R. E. Brawley, V. D. Cheyne, **M. L. Deakins*, S. B. Finn, B. J. Frey, L. H. Garrison, W. E. Hahn, H. C. Hodge, Paul Lalonde, Marian L. LeFevre, R. S. Manly, H. J. Sedwick, J. F. Volker, R. S. Voorhees.—16.

25. *St. Louis Section.*—R. F. Bleiker, William Bauer, L. R. Boling, E. P. Brady, O. W. Brandhorst, W. B. Gurley, Edward Hampp, E. H. Keys, W. E. Koch, B. E. Lischer, L. R. Main, E. B. Owen, T. E. Purcell, H. R. Raper, **H. B. G. Robinson*, R. C. Wheeler, J. D. White.—17.

26. *San Francisco Section.*—G. L. Bean, Hermann Becks, W. C. Fleming, J. R. Gill, P. J. Hanzlik, G. M. Hollenbeck, G. A. Hughes, A. J. Ker, J. A. Marshall, G. S. Millberry, R. W. Rule, W. B. Ryder, Jr., F. W. Schubert,

* Section Secretary.

E. W. Schultz, Nina Simmonds, **L. B. Taber*, M. L. Tainter, W. W. Wainwright, R. C. Zeisz.—19.

27. *Toronto Section.*—H. K. Box, A. J. Broughton, Thomas Cowlings, **A. W. Ellis*, G. W. Grieve, F. C. Husband, A. D. Mason, A. J. McDonagh, E. W. Paul, H. S. Thomson.—10.

28. *Vienna Section.*—Arwed Berg, Karl Breitner, **Fritz Driak*, Richard Grohs, Otto Hofer, Hermann Mathis, Hans Pichler, Otto Preissecker, Franz Schön Bauer, A. M. Schwarz, Harry Sicher, Karl Spring, Richard Trauner, Hermann Wolf.—14.

29. *Washington Section.*—F. A. Arnold, J. E. Ash, Ronald Barber, J. R. Beall, C. W. Camalier, A. B. Crane, H. T. Dean, B. E. Erickson, H. E. Harvey, Ales Hrdlicka, L. G. Jordan, Harry Kaplan, Henry Klein, R. A. Keilty, **H. W. Krogh*, D. F. Lynch, J. B. Mann, S. V. Mead, G. C. Paffenbarger, I. C. Schoonover, Wilmer Souder, E. R. Stone, W. T. Sweeney.—23.

30. *Winnipeg Section.*—***E. R. Bier*, G. A. Buchanan, F. T. Cadham, M. H. Garvin, K. M. Johnson, H. J. Merkeley, W. J. Riley.—7.

31. *Not now members of a section.—Amsterdam.*—T. E. de Jonge-Cohen, Elkan Sanders. *Boston.*—Benjamin Tishler. *Buffalo.*—Mathew Podolin. *Canal Zone.*—J. L. Bernier. *Chicago.*—J. S. Shell. *Denver.*—K. K. Cross. *Fort Hancock.*—R. W. Leigh. *Germany.*—Gustav Korkhaus, Friederich Proell, Rudolph Weber. *Hartford.*—R. L. Coleman. *Johannesburg.*—S. F. Gottlich. *Korea.*—J. L. Boots. *Liverpool.*—G. G. Macphee. *Little Rock.*—S. N. Blackberg. *Milan.*—Gyula Csernyei, Silvio Palazzi. *Milford.*—Aaron Isaacs. *Montevideo.*—F. M. Pucci, J. C. Sartori. *New York.*—H. W. Gillet. *New Zealand.*—C. D. M. Day, R. E. T. Hewat, R. M. S. Taylor. *Omaha.*—V. E. Levine. *Oslo.*—Ingjold Reichborn, R. F. Sognnaes. *Palestine.*—Heinrich Berger, Hugo Brasch, Berhard Gottlieb, Ernst Kellner, Samuel Lewin-Epstein, Ervin Mansbach, Abraham Sussman, Jacob Yardeni. *Philadelphia.*—Anna M. Nichols. *Pittsburgh.*—Faith P. Hadley. *Providence.*—A. L. Midgley. *St. Louis.*—G. B. Winter. *San Francisco.*—L. P. Hartley, Martha R. Jones, E. H. Mauk, F. V. Simonton. *Saranac Lake.*—W. E. Taylor. *Stockholm.*—G. Y. Hildebrand. *Swarthmore.*—J. A. Detlefson. *Toledo.*—T. E. Moore. *Tucson.*—Margaret C. Smith. *Zurich.*—Walter Hess, Maurine Willman Müller.—52.

32. *Address as of Dec. 10, 1939, unknown.*—Guido Fischer.—.

Total number of members—495; average membership of 30 sections—15.

* Section Secretary.

REGARDING THE PROPOSED NEW PLAN OF DENTAL EDUCATION AT HARVARD

During the past year a Harvard University committee has been studying the problems of dental education. The report of this committee has been considered by the Faculty of Medicine and by the University Administration.

No official statement regarding the suggestions of the committee has yet been released. Nevertheless, various accounts, based entirely upon rumor, have appeared. It is unfortunate, both for the Harvard Dental School and for dental education, that many irresponsible and misleading statements have been made which, in large part, are without foundation in fact.

It is not possible to release the details of the plan which has been formulated at this time, but it is expected that a full account of it will be made public not later than January first. In the meantime the following observations may serve to correct some of the many misapprehensions that now exist.

1—Harvard is not "going to end its dental school after seventy years".

2—It is not true that, beginning this fall, the Harvard Dental School would cease to exist. As a matter of fact the Harvard Dental School did accept this fall the usual first year class, with a full quota of students, and will carry this class through the entire four years under the present framework.

3—There is no truth in the statement, as applied to the present situation, or to the contemplated new plan, that "All candidates contemplating the study of dentistry must first enroll and qualify by acquiring the degree of doctor of medicine, before entering upon the study of dentistry".

4—The statement, that the objective of the new course in dentistry will be not to train men for the general practice of dentistry, is misleading. Whatever new plan is adopted, it will still be possible for men to qualify for general dental practice and to satisfy requirements for licensure.

5—The statement has been made that Harvard is going to discontinue teaching prosthetic and other forms of restorative dentistry, and confine itself simply to preparing men for oral surgery and other specialties. Again, this is wholly without foundation in fact.

6—The dental profession may rest assured that any modifications in the curriculum now under consideration will, if put into effect, be expected to elevate the importance of dentistry as a profession, and neither to lower its standards nor to diminish its effectiveness.

7—Until a full account of the plans of the new course has been presented officially, we ask the many who are interested in the Harvard Dental School and in the progress of dental education to delay judgment.

LEROY M. S. MINER,
Dean, Harvard Dental School

INDEX

Volume 18: 1939[1]

ACID: neutralizing power; saliva; diet, 409
——: production; lactobacilli, 513
——: ——; lactobacilli and streptococci, 266
See also under pH.
Actinomyces-like organism; pulp, 267
American College of Dentists: Wm. J. Gies awards and fellowships, 271
Age: caries incidence, 43
——: tooth; caries, 283
Alkaline reserve: saliva: decalcification, 268
ALLEN, F.: periodontitis; bacterial counts, 253
Alveolalgia: analysis, 256
Anacoresis: periapical inflammation, 527
Anesthesia: mandibular block, 561
ANGE, L. E.: density; teeth, 203
Anomaly: dental; diet, 284
——: teeth, 280
Anthrax bacilli: protective effect of saliva, 278
APPLEBAUM, E.: hypophysectomy; effect on developing teeth, 287
APPLETON, J. L. T.: anthrax bacilli; protective effect of saliva, 278
ARCHER, W. H.: alveolalgia; case analyses, 256
——: Horace Wells; chronological history, 278
Ascorbic acid: determination, 273
See also vitamin C.
Attrition: caries and crowding; Eskimo, 255
Awards: Wm. J. Gies, 271

B. ACIDOPHILUS. *See under* Lactobacilli.
Bacteria: carbohydrate degradation, 261
——: caries, 185, 177
——: incidence in periodontitis, 253
——: influence of saliva, 214
——: oral; variations from time to time, 61
See also under various organisms.
Bacteriology: radiography; pulp involved teeth, 286
BALE, W. F.: phosphorus metabolism; bones and teeth; radioactive isotope, 265
BECKS, H.: resting saliva; rate of flow, 431
——: salivary analyses; biometric evaluation, 1
——: salivary flow; effect of activation, 447
BERMAN, M.: hyperpyrexia; effect on tooth, 239
BERNSTEIN, J. G.: short wave radiation; effect on tooth, 247
BEVELANDER, G.: dentinogenesis; diet and hormones, 533
——: short wave radiation; effect on tooth, 247
BIBBY, B. G.: lysozyme; saliva; purification, 242
——: oral flora; variations; time to time, 61
——: oral lactobacilli and streptococci; acid production, 266
Biometry: evaluation; salivary analyses, 1
Biscuit: pilot; caries, 343
Bite raising: temporomandibular reactions, 279
BLAYNEY, J. R.: caries; lactobacilli; quantitative estimation, 260

[1] Abbreviations—I. A. D. R.: International Association for Dental Research, J. D. Res.: Journal of Dental Research.

Blood: dyscrasias; oral symptoms, 268
——: groups; students, 247
BODECKER, C. F.: fluorescence; vital and pulpless teeth, 265
BOLING, L. R.: this index, 571
Bone: phosphorus; radioactive isotope, 265
——: calcification, 143
——: effect of thyro-parathyroidectomy, 251
BOYD, J. D.: caries; *b. acidophilus*, 185
BRADEL, S. F.: caries; lactobacilli; quantitative estimation, 260
BRASCH, H.: tooth fusion; cementum, 277
Breath: odor, 127, 255, 355
BREITNER, C.: temporomandibular reactions; bite raising, 279
BRENING, R. H.: breath; odor, 127, 255, 355
——: halitosis; cryoscope and osmoscope, 127
BREUER, K.: paradental disease; ascorbic acid determination, 273
——: ——; prevention, 272
——: ——; vitamin C, 272
Bridges: fixed-removable, 275
BRODIE, A. G.: cephalometry; orthodontia, 240
BROOMALL, A.: phosphatase; saliva, 254
BROWN, G. N.: diet; dental anomalies, 284
BURWASSER, P.: diet; effect on gingiva, 237, 389

CALCIFIED tissue: density, 203, 237, 557
Calcium: soluble; caries, 259
——: teeth, 547
See also under saliva.
Calcium hydroxide: effect on pulp, 263, 373
Calculus: saliva; Eskimo, 244
CAMPAIGNE, E.: pH; isolated mouth areas, 261
Carbohydrates: caries, 343
——: degradation by bacteria, 261
——: from lactobacilli; serological reactions, 259
See also under sugar.
Carbon dioxide; teeth, 547
Caries: artificial; *S. viridans*, 281
——: attrition and crowding; Eskimo, 255
Caries (*con.*):
——: calcification, 143
——: dentin composition, 266
——: Eskimo, 244, 255, 262, 343
——: fats; vitamin D, 189
——: fluorine, 252, 481
——: hypoplasia, 257
——: incidence; age and sex, 43
——: lactic acid content, 259
——: lactobacilli, 185
——: ——; effect of sugars, 262
——: ——; estimation, 242
——: nutrition, 143, 189, 244, 252, 255, 262, 343, 409
See also under diet.
——: pilot biscuit, 343
——: proximal surfaces, 261
——: rat; observation, 153, 262
——: ——; recording, 469
——: saliva, 238, 246, 257, 285, 395
——: ——; Eskimo, 244, 255
——: streptococci, 177
——: tooth age, 283
——: tooth hardness, 265
——: Tristan da Cunha, 243
Caries-free children: lactobacilli, 497
CART, J. F.: anomalies of the teeth, 280
Casting: gold; effect of variables, 249
Catalase: saliva, 238
Cement: silicate; chemical reaction, 250
Cementum: discovery, 239
——: tooth fusion, 277
Cephalometry: orthodontics, 240
Chemotaxis: saliva; leukocytes, 361
CHEYNE, V. D.: salivary glands; rat, extirpation, 280, 457
Chillingworth, F. P.: necrology, 288
Classification: lactobacilli, 513
Composition: dentin; caries, 266
——: precolumbian teeth, 547
COOK, T. J.: blood dyscrasias; oral symptoms, 268
COX, G. J.: caries; fluorine, 481
——: ——; recording in rats, 469
——: method; grinding rat molars, 153, 262
Crosby, A. W.: necrology, 289

Crowley, M. C.: actinomyces-like organism; pulp, 267
——: pulp involved teeth; bacteriologic and radiographic findings, 286
Cryoscope: halitosis, 127, 255, 355
Csernyei, J.: focal infection: diagnosis and incidence, 274
——: periapical inflammation; anacoresis, 527
Cusp: form; artificial teeth, 249
Cyst: dentigerous, 280

Davidson, S.: caries; proximal surfaces, 261
Deakins, M. L.: density; calcified tissue, 237, 557
Decalcification: enamel; orthodontic bands, 264
——: ——; saliva, 268
——: lactobacilli, 525
Degradation: carbohydrates, 261
Density: calcified tissues, 203, 237, 557
Dentifrice: detergents; toxicity, 81
Dentin: apposition rate, 91, 161
——: composition, 547
——: ——; caries, 266
——: hardness, 265
——: 16μ rhythm, 91, 161
——: Tomes granular layer, 274
Dentinogenesis: diet and hormones, 553
——: effect of fever, 239, 247, 252
Denton, G. B.: cementum; discovery, 239
Denture: acrylic resin; effect on oral health, 250
——: plastics, 248, 381
——: ——; physical properties, 248
Detergents: toxicity, 81
Development: bones and teeth; effect of thyro-parathyroidectomy, 251
——: dental; facial growth, 241
——: dentin; diet and hormones, 553
——: tooth, 91, 161, 287
——: tooth germs; transplanted, 253
Diabetes: tooth loss, 245
Diagnosis: biochemical index; saliva, 283
——: erythema multiforme; Vincent's infection, 281
Diagnosis (*con.*):
——: focal infection, 274
Diet: caries, 143, 189, 244, 252, 255, 262, 343, 409
——: ——; Eskimo, 244, 255, 262, 343
——: dental anomalies, 284
——: dentinogenesis, 533
——: effect on gingiva, 237, 389
——: fluorine. *See under* fluorine.
——: hardness; caries, 343
——: ——; effect on gingiva, 237, 389
——: periodontal disease, 272, 273, 305
——: saliva; acid neutralizing power, 409
Dietz, A. K.: anthrax bacilli; protective effect of saliva, 278
——: saliva; chemotaxis; leukocytes, 361
Disease: blood; oral symptoms, 268
——: systemic; caries, 257
See also under various diseases.
Dixon, S. F.: caries; fluorine, 481
——: ——; recording in rats, 469
——: method; grinding rat molars, 153, 262
Dodds, M. L.: caries; fluorine, 481
——: ——; recording in rats, 469
Dogs: epithelial attachment, 287
Downs, W. B.: cephalometry; orthodontia, 240
Drain, C. L.: caries; *b. acidophilus*, 185
Dry socket. *See* alveolalgia.
Dunning, J. M.: caries; age and sex incidence, 43

Easton, G. S.: gold color wrought metal for technic use, 281
Eckhardt, J.: precolumbian teeth; composition, 547
Education: Harvard dental school, 570
Electric currents: intra-oral, 240
Enamel: apposition rate, 91, 161
——: composition, 547
——: decalcification; orthodontic bands, 264
——: ——; saliva, 268
——: density, 203
——: hardness, 265
——: 16μ rhythm, 91, 161
——: solubility, 238, 260

Epithelial attachment: dogs, 287
See also periodontal disease.
Eruption: molar; trauma, 267
——: rat incisors; effect of fluorine, 253
Erythema multiforme: diagnosis, 281
Eskimo: calculus; caries and saliva, 244
——: caries; attrition and crowding, 255
——: ——; nutrition, 244, 255, 262, 343
Extraction: incidence in diabetes, 245
——: incidence; alveolalgia, 256

FATS: caries, 189
Fellowships: Wm. J. Gies research, 271
Fever: effect on tooth, 239, 247, 252
FIGG, W. A.: traumatic changes; impacted canine, 267
Fillings: temporary; sealing agents, 67
FINN, S. B.: caries; fluorine, 252
Firing method: porcelain, 285
Fluorescence: pulpless and vital teeth, 265
Fluorine: caries, 252, 481
——: effect on eruption, 253
——: relative toxicity, 491
Focal infection: diagnosis and incidence, 274
FORBES, J. C.: saliva; acid neutralizing power; diet, 409
FOSDICK, L. S.: breath; odor, 127, 355
——: carbohydrates; degradation by mouth organisms, 261
——: enamel; solubility in saliva; pH levels, 260, 417
——: halitosis; cryoscope and osmoscope, 127
——: pH; isolated mouth areas, 261
FRENCH, E. L.: precolumbian teeth; composition, 547
FREY, B. J.: eruption rat incisors; effect of fluorine, 253
Fusing: dental porcelain, 282
Fusion: cementum, 277

GARRISON, L. H.: fever; effect on rat incisor, 252
GIELER, C. W.: denture plastics; physical properties, 248, 381
GIES, W. J.: endowment fund, 296, 297
——: introduction of, 225, 272
——: research fellowships, 271
GIES, W. J.: inaugural address; I. A. D. R., 230, 272
Gingiva: effect of diet, 237, 305, 389
Gingivitis: hormone therapy, 329
Gland: mucous; hypertrophy, 284
Gold: casting; effect of variables, 249
GOLDSTEIN, A.: cephalometry; orthodontia, 240
GOLDSTEIN, H.: erythema multiforme; Vincent's infection; differential diagnosis, 281
GOLDSWORTHY, N. E.: lactobacilli, classification, 513
GORE, J. T.: saliva; alkaline reserve; enamel decalcification, 268
GOTTLIEB, B.: Tomes granular layer, 274
Granuloma: anacoresis, 527
GRIMMER, E. A.: trauma; erupting premolar, 267
GROSSMAN, L. I.: temporary fillings; sealing agents, 67
Growth: facial; dental development, 240, 241

HACKH, I. W. D.: necrology, 290
HAHN, W. E.: tooth germs; heterotopic development, 253
Halitosis. See breath.
HAMMOND, C.: artificial caries; viridans, 281
——: dissociating streptococci; caries, 177
Hardness: dentin and enamel, 265
——: food; caries, 343
——: ——; gingiva, 237, 389
HARRISON, R. W.: carbohydrates from oral lactobacilli; serological reactions, 259
HARTLEY, G. F.: caries; lactobacilli; quantitative estimation, 260
Harvard Dental School: plan, 570
HATTON, E. H.: I. A. D. R.; list of members, 566
Health: oral; effect of acrylic resins, 250
——: ——; effect of cusp form, 249
HEMMENS, E. S.: carbohydrates from oral lactobacilli; serological reactions, 259
HIGLEY, L. B.: dental development; facial growth, 241

HILL, T. J.: address; I. A. D. R.; saliva; influence on bacteria, 214, 271
——: diet; effect on gingiva, 237, 389
HINE, M. K.: oral flora; variations; time to time, 61
Histology: oral mucosa, 256
See also dentin, enamel, pulp, teeth, etc.
History: cementum, 239
——: Horace Wells, 278
HODGE, H. C.: caries; fluorine, 252
——: density; calcified tissues, 133, 203
——: microhardness; carious and noncarious teeth, 265
——: precolumbian teeth, composition, 547
HOFFMAN, M. M.: apposition rate; dentin and enamel, 161
——: 16μ rhythm; dentin and enamel; fish to man, 91
——: vitamin A deficiency; rat incisor; radiographic and histologic changes, 263
HOLLANDER, F.: caries; age and sex incidence, 43
Hormones: therapy; gingivitis, 329
——: dentinogenesis, 533
HOSKINS, M. M.: dentinogenesis; diet and hormones, 533
HYNEK, L.: vitamin C; paradental disease, 272
Hypertrophy: mucous gland, 284
Hypophysis: removal; effect on developing teeth, 287
Hypoplasia: caries, 257

I A. D. R.: executive proceedings, 294
——: list of officers and new members, 301
——: proceedings; address of retiring president, 214, 271
——: ——; contents, 213
——: ——; inaugural address, 230, 272
——: ——; index of participants, 236
——: ——; J. D. Res., 296
——: ——; president-elect; introduction, 225, 272
——: ——; report of necrology committee, 288
——: register of members, 566
I. A. D. R. (*con.*):
——: reports of officers and committees, 294
Impacted canine: traumatic changes, 267
Incidence: alveolalgia, 256
——: caries; age and sex, 43
——: focal infection, 274
——: oral tuberculosis, 287
Index: this journal, 571
Inflammation: periapical; anacoresis, 527
Isotope phosphorus; bones and teeth, 265

J. D. RES.: committee reports, 296
——: Gies endowment fund, 297
——: index, 571
——: sustaining subscribers, 565

KALDARAR, H.: paradental disease; ascorbic acid determination, 273
KANNER, O.: galvanic currents; intra-oral, 240
KARSHAN, M.: calcification; influence of minerals, protein and sugar, 143
——: calculus, caries and saliva; Eskimo, 244
——: caries; fats; vitamin D, 189
——: ——; salivary factors, 395
——: dietary studies; Eskimo children, 244
KARSNER, H. T.: research; rein and spur, 269
Kemple, F. C.: necrology, 291
KLAFFENBACH, A. O.: fusing; dental porcelains, 282
KLEIN, H.: tooth age; caries, 283
KNIGHTON, H. T.: monilia and yeasts; oral cavity, 103
——: yeastlike organisms; significance, 258
KOCH, W. E.: this index, 571
KOKATNUR, V. R.: saliva; caries susceptibility, 238
KRASNOW, F.: salivary analysis; biochemical diagnostic index, 283

LACTOBACILLI: acid production, 266, 513
——: carbohydrates; serological reactions, 259
——: caries, 185
——: ——; effect of sugars, 262

Lactobacilli (*con.*):
——: ——; estimation, 260
——: caries-free children, 497
——: classification, 513
——: decalcifying ability, 525
——: estimation, 242
Lactic acid: content; caries, 259
Lane, C.: necrology, 291
LASKOWSKI, B. S.: cusp form; artificial teeth, 249
LEFEVRE, M. L.: phosphorus metabolism; bones and teeth; radioactive isotope, 265
LEFKOWITZ, W.: enamel decalcification; orthodontic bands, 264
LEONARD, H. J.: saliva; caries, 238
Leukocytes: saliva; chemotaxis, 361
LEVINE, V. E.: caries; attrition and crowding; Eskimo, 255
Lysozyme: saliva; purification, 242

MANDIBLE: measurements of ramus, 561
MANLY, R. S.: caries; changes in dentin composition, 266
——: density; calcified tissue, 133, 203, 237, 557
Materials: temporary fillings; sealing agents, 67
See also cement, denture plastics, etc.
MATUSCHAK, M. C.: caries; fluorine, 481
——: ——; recording in rats, 469
McCREA, M. W.: sustaining subscribers, J. D. R., 565
McDowell, A. R.: necrology, 292
MERRITT, A. H.: committee on endowment, J. D. Res, 296
Metal: technic; gold color, 281
Method: density; calcified tissue, 237, 557
——: estimation of lactobacilli, 260
——: grinding rat molars, 153
——: halitosis, 127
——: lactobacilli estimation, 243
——: recording caries; rats, 469
MIDGLEY, A. L.: Wm. J. Gies awards and research fellowships, 271
MILLER, B. F.: caries; lactic acid content, 259
MINER, L. M. S.: Harvard dental school, 570
Minerals: calcification, 143
Monilia: oral cavity, 103, 258
MOON, H. S.: caries; proximal surfaces, 261
Mucosa: oral; histology, 256
MUNTZ, J. H.: caries; lactic acid content, 259
MYER, E.: cephalometry; orthodontia, 240
MYERS, R. E.: gold castings; effect of variables, 249

NECROLOGY, 288
Neoprontosil: phagocytic activity, 237
NEUMANN, F.: fixed-removable bridges, 275
——: prosthetic treatment: mandibular prognathism, 275
NEUWIRT, F.: paradentosis, prevention, 276
——: pulp treatment, 276
NEUWIRTH, I.: diet; dental anomalies, 284
Nutrition: caries, 143, 189, 244, 252, 255, 262, 343, 409
——: periodontal disease, 272, 273, 276, 305
See also diet.

OARTEL, J. S.: blood groups; dental students, 247
——: syphilis; incidence; dental students, 247
Occlusion: crowding; Eskimo, 255
Odor: breath, 127, 255, 355
ORBAN, B.: saliva; cellular elements; caries, 258
Orthodontic bands: enamel decalcification, 264
Orthodontics: cephalometry, 240
Osmoscope: halitosis, 127

PALMER, C. E.: tooth age; caries, 283
Paradental disease. *See* periodontal disease.
Parathyroid: thyroid; removal; effect on bones and teeth, 251
PENDLETON, E. C.: oral mucosa; histology, 256
Periodontal disease: ascorbic acid, 273
——: bacterial counts, 253
——: diet, 272, 273, 276, 305
——: prevention, 272, 276

Peroxide compounds: saliva, 238
PERRINE, R. L.: detergents; toxicity, 81
PFEIFFER, K. R.: gold castings; effect of variables, 249
pH: saliva; enamel solubility, 260
——: isolated mouth areas, 261
Phagocytosis, 237
Phosphatase: saliva, 254
Phosphorus: metabolism; bones and teeth; radioactive isotope, 265
——: soluble; caries, 259
——: teeth, 547
Plastics: denture, 248, 381
pO: breath, 355
Porcelain: fusing, 282
——: physical properties; firing methods, 285
Prevention: periodontal disease, 272, 276
PRICE, W. A.: caries; saliva, 246
Prognathism: prosthetic treatment, 275
Prosthetic treatment: prognathism, 275
Protein: calcification, 143
Pulp: actinomyces-like organism, 267
——: amputation, 263, 264, 373
——: effect of fever, 252
——: effect of short-wave radiation, 247
——: reaction; calcium hydroxide, 263, 373
——: treatment, 263, 264, 276, 373
Pulp involved teeth: bacteriologic and radiographic findings, 286

RADIATION: short-wave; effect on tooth, 247
Radiography: bacteriology; pulp involved teeth, 286
RADUSCH, D. F.: periodontal conditions; diet, 305
Rat: salivary glands, 280, 457
RATHBUN, M.: precolumbian teeth; composition, 547
REED, E.: blood groups; dental students, 247
——: syphilis; incidence; dental students, 247
Research: fellowships; Wm. J. Gies, 271
——: rein and spur, 269
Resorption: deciduous teeth, 73, 254
Rickert, U. G.: necrology, 293
ROBINSON, H. B. G.: hypertrophic palatine mucous gland, 284
——: I. A. D. R.; proceedings, 213
——: this index, 571
ROGERS, W. M.: deciduous teeth; shedding, 73, 254
ROSEBURY, T.: calcification; influence of minerals, protein and sugar, 143
——: caries; fats; vitamin D, 189
——: ——; pilot biscuit, 343
RZESZOTARSKI, J. S.: pulpectomy and amputation; histologic findings, 264

SALIVA: acid neutralizing power; diet, 409
——: activated, 441
——: alkaline reserve; enamel decalcification, 268
——: analysis; diagnostic index, 283
——: biometric evaluation; analyses, 1
——: calculus and caries, 244
——: caries, 238, 246, 257, 395
——: ——; tests, 285
——: caries-free children, 497
——: cellular elements; caries, 258
——: chemotaxis; leukocytes, 361
——: influence on bacteria, 214, 278
——: lysozyme, 242
——: pH; enamel solubility, 260
——: phosphatase, 254
——: protective influence, 278
——: rate of flow, 431, 441, 447
——: resting, 431
Salivary calculus. *See* calculus.
Salivary glands: rat; extirpation, 280, 457
SALMON, T. N.: thyro-parathyroidectomy; effect on development, 251
SARTORI, J. C.: dental porcelain; physical properties; firing methods, 285
SCHAINIS, M. E.: caries; saliva tests, 285
SCHOONOVER, I. C.: silicate cements; chemical reactions, 250
SCHOUR, I.: apposition rate; dentin and enamel, 161
——: 16μ rhythm; dentin and enamel; fish to man, 91

SCHOUR, I. (*con.*):
——: vitamin A deficiency; rat incisor; radiographic and histologic changes, 263
SCHUCK, C.: fluorine; relative toxicity, 491
SEGENREICH, A.: diet; dental anomalies, 284
SHAPIRO, H. H.: deciduous teeth; shedding, 73, 254
Shedding: deciduous teeth, 73, 254
SIEGEL, E. H.: calculus, caries and saliva; Eskimo, 244
——: dietary studies; Eskimo children, 244
Silicate cement: chemical reaction, 250
SKINNER, E. W.: denture plastics; physical properties, 248, 381
SMITH, M. C.: vitamin A deficiency; rat incisor; radiographic and histologic changes, 263
SNYDER, M. L.: lactobacilli; caries-free children, 497
——: ——; method of estimation, 242
SOGNNAES, R. F.: dental study; Tristan da Cunha, 243
Solubility: enamel, 238, 260
SOMMER, R. F.: pulp involved teeth; bacteriologic and radiographic findings, 286
SOUDER, W.: silicate cements; chemical reactions, 250
SPEIDEL, T. D.: caries; *b. acidophilus*, 185
——: dental development; facial growth, 241
STARKE, A. C.: enamel; solubility in saliva; pH levels, 260, 417
Statistical methods: evaluation; salivary analyses, 1
STEIN, G.: caries; hypoplasia, 257
STILL, J. L.: lactobacilli; classification, 513
Streptococcus: acid production, 266
——: dissociating; caries, 177
——: phagocytosis, 237
Streptococcus viridans: caries, 281
Students: dental; blood groups, 247
——: ——; syphilis incidence, 247
Sugar: calcification, 143
——: caries; lactobacilli, 262
Sulfanilamide: phagocytic activity, 237
See also neoprontosil.
SULLIVAN, H. R.: lactobacilli; classification, 513
——: ——; decalcifying ability, 525
SULZER, G. F.: breath; odor, 127, 255, 355
——: halitosis; cryoscope and osmoscope, 127
SUSSMANN, A.: Tomes granular layer, 274
——: tooth fusion; common cementum, 277
SWANSON, W. F.: blood groups; dental students, 247
——: syphilis; incidence; dental students, 247
Syphilis: incidence in dental students, 247
SZOKÓLOCZY-SYLLABA, B.: mandible; measurements of ramus, 561

TAINTER, M. L.: detergents; toxicity, 81
TAYLOR, W. E.: oral tuberculosis; incidence, 287
Teeth: artificial; cusp form, 249
——: calcification, 143
——: deciduous; shedding, 73, 254
——: effect of thyro-parathyroidectomy, 251
——: phosphorus; radioactive isotope, 265
——: precolumbian; composition, 547
TEFFT, H.: precolumbian teeth; composition, 547
Temporomandibular articulation: bite raising, 279
THRONDSON, A. H.: detergents; toxicity, 81
Thyroid: parathyroid; removal; effect on bones and teeth, 251
Transplantion: tooth germs; development, 253
Trauma: erupting premolar, 267
——: impacted canine, 267
Treatment: gingivitis; hormones, 329
——: pulp, 263, 264, 276, 373
Tristan da Cunha: dental study, 243
TOLLER, J. R.: epithelial attachment; dogs, 287
Tomes granular layer, 274
Toxicity: detergents, 81
——: fluorine, 491
Tuberculosis: oral; incidence, 287

TUNNICLIFF, R.: sulfanilamide and neoprontosil; phagocytic activity, 237

VAN HUYSEN, G.: caries; proximal surfaces, 261
VAN KESTEREN, M.: lactobacilli and streptococci; acid production, 266
——: lysozyme; saliva; purification, 242
VAN KIRK, L. E.: diabetes; tooth loss, 245
Vincent's infection: diagnosis, 281
Vitality: difference in fluorescence, 265
Vitamin: A; effect on rat incisor, 263
——: C; paradental disease, 272
See also ascorbic acid.
——: D; caries, 189
VOLKER, J. F.: enamel; solubility studies, 238

WACHMANN, K., SR.: necrology, 294
WAINWRIGHT, W. W.: activated saliva; rate of flow, 441
——: salivary analyses; biometric evaluation, 1
——: salivary flow; effect of activation, 447
WALKER, W. E.: caries; fluorine, 481
WAUGH, D. B.: lactobacilli; caries; effect of sugars; Eskimo, 262
WAUGH, L. M.: calculus, caries and saliva; Eskimo, 244
——: dietary studies; Eskimo children, 244
——: Gies endowment fund, 297
——: introduction of president-elect; I. A. D. R., 225, 272
WAUGH, L. M. (*con.*):
——: lactobacilli; caries; effect of sugars; Eskimo, 262
WEINMANN, J. P.: saliva; cellular elements; caries, 258
WELCH, E.: precolumbian teeth, composition, 547
Wells, Horace: chronological history, 278
WESSINGER, G. D.: carbohydrates; degradation by mouth organisms, 261
WHITEHEAD, E. R.: hypertrophic palatine mucous gland, 284
WILLS, J. H.: saliva; acid neutralizing power; diet, 409
WOLF, E. A.: phosphatase; saliva, 254
WRIGHT, W. H.: acrylic resin dentures; oral health, 250

YEASTLIKE organisms: significance, 258
Yeasts: oral cavity, 103

ZANDER, H. A.: pulp; reaction to calcium hydroxide, 263, 373
ZIDEK, Z. C.: carbohydrates from oral lactobacilli; serological reactions, 259
ZISKIN, D. E.: hormonal therapy; gingivitis, 329
——: hypophysectomy; effect on developing teeth, 287
——: thyro-parathyroidectomy; effect on development, 251

Volume 18 Number 6

JOURNAL of DENTAL RESEARCH

JAN 15 1940

FOUNDED BY WILLIAM J. GIES IN 1919

Official Publication of the

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

December, 1939

Experimental dental caries. III. A system of recording corn meal caries in rats. *Gerald J. Cox, Mary L. Dodds, Sara F. Dixon,* and *Margaret C. Matuschak* (Mellon Institute).... 469

Experimental dental caries. IV. Fluorine and its relation to dental caries. *Gerald J. Cox, Margaret C. Matuschak, Sara F. Dixon, Mary L. Dodds,* and *W. E. Walker* (Mellon Institute)..... 481

A study of the relative toxicity of sodium fluoride in a dry diet, in milk and in water. *Cecilia Schuck,* Ph.D. (Purdue University)..... 491

The occurrence of lactobacilli and other acidogenic organisms in the saliva of selected caries-free children. *Marshall L. Snyder* (University of Michigan)..... 497

Acid-production as a basis for classifying lactobacilli from carious teeth. *H. R. Sullivan, J. L. Still,* and *N. E. Goldsworthy* (University of Sydney, Australia)..... 513

A comparison of the decalcifying ability of different types of oral lactobacilli. *H. R. Sullivan* (University of Sydney, Australia)..... 525

Anacoric effect of chronic periapical inflammations, *Julius Csernyei,* M.D. (Milan, Italy)... 527

A comparison between dietary and hormonal factors in the development of dentin. *Gerrit Bevelander* and *Margaret M. Hoskins* (New York University)..... 533

Composition of pre-columbian teeth. *Ethel L. French,* Ph.D., *Helen Tefft, Margaret Rathbun, Janet Eckhardt, Eileen Welch* and *Harold C. Hodge,* Ph.D. (University of Rochester)... 547

A method for microdetermination of density in calcified tissues. *Martin Deakins,* D.D.S., M.S. and *Richard S. Manly,* Ph.D. (University of Rochester)..... 557

Measurements of the ascending ramus of the mandible. *Béla Szokóloczy-Syllaba,* Dr. med. (Royal Hungarian University, Budapest)..... 561

Supplement

I. Sustaining subscribers to the Journal of Dental Research. Compiled by the *Circulation Manager*..... 565

II. International Association for Dental Research. Register of members by sections as of December 10, 1939. Compiled by the *General Secretary*..... 566

III. Proposed new plan of dental education at Harvard. *L. M. S. Miner*..... 570

Index to Volume 18..... 571

General Matter for Volume 18:

Title page..... i

Contents of volume..... ii

PUBLISHED BI-MONTHLY AT MT. ROYAL AND GUILFORD AVENUES, BALTIMORE, MD.
By THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

EDITORIAL OFFICE: 4559 Scott Ave., St. Louis, Mo.

Copyright, 1939, by the International Association for Dental Research

Made in United States of America

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

EDITORS OF THE JOURNAL OF DENTAL RESEARCH

Editor Emeritus—William J. Gies

Publication Committee: J. Frank Hall, M. W. McCrea and Hamilton B. G. Robinson.

Editor—Hamilton B. G. Robinson. *Business Manager*—J. Frank Hall. *Circulation Manager*—M. W. McCrea.

Assistants to the editor—L. R. Boling and W. E. Koch, Jr. *Assistant to the business manager*—R. L. Simpson, Jr. *Assistant to the circulation manager*—M. S. Aisenberg.

Board of Editors—Editor-emeritus, members of the Publication Committee and sectional representatives named in fifth column below.

THE SECTIONS—THEIR SECRETARIES, AND REPRESENTATIVES IN THE COUNCIL AND IN THE BOARD OF EDITORS (1937–'38)

Division	*Section*	*Secretary*	*Councillor*	*Editor*
Canada	Halifax	S. G. Ritchie	J. S. Bagnall	S. G. Ritchie
	Toronto	A. W. Ellis	A. J. McDonagh	A. J. McDonagh
	Winnipeg	E. Roy Bier	H. J. Merkeley	E. Roy Bier
China	Chengtu	R. Gordon Agnew	R. Gordon Agnew	R. Gordon Agnew
England	London	Evelyn Sprawson	Evelyn Sprawson	Evelyn Sprawson
Germany	Prague	Friedr. Neumann	Friedr. Neumann	Jan Jesensky
	Vienna	Fritz Driak	R. Trauner	F. Shönbauer
Hungary	Budapest	Ferenc Köszeg	———	———
South Africa	Johannesburg	W. A. Wilson	Geoffrey Friel	J. C. M. Shaw
United States	Ann Arbor	Philip Jay	Philip Jay	Philip Jay
	Baltimore	M. S. Aisenberg	M. S. Aisenberg	A. H. Schultz
	Boston	F. W. Morse	L. M. S. Miner	P. E. Boyle
	Chicago	E. C. Pendleton	Isaac Schour	Edgar G. Coolidge
	Cleveland	S. W. Chase	Thomas J. Hill	S. W. Chase
	Columbus	Paul C. Kitchin	Paul C. Kitchin	C. O. Boucher
	Detroit	Samuel J. Lewis	P. C. Lowery	Samuel J. Lewis
	Houston	E. Kotanyi	E. Kotanyi	E. Kotanyi
	Iowa City	A. O. Klaffenbach	A. W. Bryan	George S. Easton
	Louisville	Raymond E. Myers	Grant Van Huysen	H. T. Knighton
	Minnesota	Harold G. Worman	P. J. Brekhus	J. T. Cohen
	New Haven	B. G. Anderson	———	———
	New Orleans	C. S. Tuller	S. L. Tiblier	F. J. Wolfe
	New York	Isaac Neuwirth	L. M. Waugh	Theodor Rosebury
	Philadelphia	Thomas J. Cook	Thomas J. Cook	J. L. T. Appleton
	Pittsburgh	L. E. Van Kirk	H. E. Friesell	L. E. Van Kirk
	Richmond	J. Frank Hall	Harry Bear	A. D. Brashear
	Rochester	Martin Deakins	Joseph Volker	Basil G. Bibby
	San Francisco	Loren B. Taber	W. C. Fleming	J. A. Marshall
	St. Louis	H. B. G. Robinson	L. R. Main	R. C. Wheeler
	Washington	H. W. Krogh	H. T. Dean	J. B. Mann
Materials Group		E. W. Skinner	W. S. Crowell	I. C. Schoonover

Subscription price $5.00 per volume. Single numbers when available $1.00.

Entered as second-class matter June 24, 1919, at the Post Office at Baltimore, Maryland, under the act of March 8, 1879

WAVERLY PRESS, INC.

BALTIMORE, U. S. A.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Organized: New York City, Dec. 10, 1920. Of those at the meeting of organization, the following remain active members: Adolph Berger, Theodor Blum, G. W. Clapp, H. S. Dunning, W. J. Gies, H. W. Gillett, R. G. Hutchinson, Jr., T. P. Hyatt, Milo Hellman, A. H. Merritt, B. B. Palmer, P. R. Stillman, L. M. Waugh.

General Meetings have been held on this schedule: (1) New York, Dec. 21, '22. (2) Chicago, Mar. 7, '24. (3) Chicago, Mar. 20, '25. (4) Chicago, Mar. 26, '26. (5) New York, Apr. 19, '27. (6) Washington, Mar. 25–28, '28. (7) Chicago, Mar. 23–24, '29. (8) Toronto, Mar. 24–26, '30. (9) Memphis, Mar. 21–22, '31. (10) Columbus, Mar. 19–20, '32. (11) Chicago, Mar. 18–19, '33; adjourned executive session, New York, May 25, '33. (12) Chicago, Mar. 17–18, '34. (13) Chicago, Mar. 16–17, '35. (14) Louisville, Mar. 14–15, '36. (15) Baltimore, Mar. 13–14, '37. (16) Minneapolis, Mar. 12–13, '38. (17) Cleveland, Mar. 18–19, '39.

Objects (*Const., Art. II*): . . . "(a) To promote broadly the advancement of active research in all branches of dental science and in related phases of the sciences that contribute directly to the development of dentistry, and which add to the knowledge of the mouth and teeth and of their relations to the body as a whole; . . . (b) to encourage and facilitate coöperative effort and achievement by, and mutual helpfulness among, investigators in all nations in every division of stomatology; (c) to the end, particularly, that dentistry may render cumulatively more perfect service to humanity." (The Association has been using "dentistry" and "stomatology" as synonyms.)

Membership: total (Dec. 10, 1939), 495. *Eligibility* (*Const., Art. 14, Sec. 1*).—"Any person who has conducted, and published an account of, a meritorious original investigation in dental science, or in any of the sciences contributory to stomatology, shall be eligible to membership in this Association, providing such person conforms to the recognized standards of professional ethics." *Election.*—New members may be elected *only at general meetings of the Association.* Membership in a section is secondary to membership in the Association as a whole. Removal of members from one geographic center to another does not affect their membership in the Association.

Endowment fund of the International Association for Dental Research (as of March 10, 1939): $1,219.36. There have been no expenditures from this fund.

Divisions may be organized by the members in any nation. There are now divisions in Canada, China, England, Germany, Hungary, South Africa, and the United States—8. *Sections* may be organized by the members in geographic centers within the divisions. There are now sections (27) in Vienna ('29); Halifax ('28), Toronto ('21), Winnipeg ('30); Chengtu ('34); Prague ('32); London ('31); Budapest ('34); Johannesburg ('34); Ann Arbor ('23), Baltimore ('33), Boston ('20), Chicago ('20), Cleveland ('30), Columbus ('32), Detroit ('37), Iowa City ('36), Louisville ('32), Minnesota ('28), New Haven ('30), New Orleans ('37), New York ('20), Philadelphia ('28), Pittsburgh ('28), Richmond ('33), Rochester ('33), St. Louis ('28), San Francisco ('24), Washington, D. C. ('31). The names of the secretaries are given on p. 2 of this cover.

Sectional meetings in 1938–1939. Ann Arbor—May. *Boston*—Jan. 9, Feb. 6, Mar. 6, May 8. *Chicago*—Oct. 20, Jan. 20, April 21, May 19. *Iowa City*—Jan. 10. *New York*—Jan. 19, June 1. *Philadelphia*—Oct. 29, Dec. 16. *Richmond*—Jan. 27. *St. Louis*—Nov. 29, Mar. 26, Dec. 10, Mar. 27, Dec. —. *Washington*—Nov. 29, Mar. 19, April 11, Dec.

Journal of Dental Research: the Association's official publication. See register of officers and editors on page 2 of cover.

The 18th General Meeting of the INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH will be held at the Benj. Franklin Hotel, Philadelphia, Pa., March 13–14, 1940; coordinated with the annual meetings of the AMERICAN ASSOCIATION OF DENTAL SCHOOLS, March 15–17, and Omicron Kappa Upsilon, March 16.

A joint program sponsored by the AMERICAN COLLEGE OF DENTISTS and participated in by the AMERICAN ASSOCIATION OF DENTAL SCHOOLS, the AMERICAN ASSOCIATION OF DENTAL EDITORS, the AMERICAN DENTAL ASSOCIATION and the INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH will be presented in Baltimore, March 17.

THE DENTAL CENTENARY CELEBRATION will be held in Baltimore, Md., March 18, 19 and 20, 1940.

BOOKS RECEIVED

Advisory Committee on Research in Dental Caries, American Dental Association, *Dental Caries* (Chicago, American Dental Association: 1939) pp. 189.

Darlington, C. G., Wilson, G. W., Miller, H. C., Wright, W. H., and Moore, G. R., *The 1939 Year Book of Dentistry* (Chicago, Year Book Publishers: 1939) pp. 799.

INFORMATION FOR PROSPECTIVE CONTRIBUTORS, SUBSCRIBERS, OR ADVERTISERS

The JOURNAL OF DENTAL RESEARCH was dedicated by its founder to the dissemination of knowledge pertaining to the teeth and mouth, and to their relations to the body as a whole. It publishes original investigations in these and related fields. Articles are accepted only on condition they are contributed solely to this JOURNAL. Articles appearing in other domestic or foreign periodicals will not be reprinted in the Journal except in extraordinary cases. The JOURNAL will supply no more than six half-tones or zinc etchings without cost to the author. References to the literature must conform to the following style:

1. (To a periodical)—Doe, J. A., *J. D. Res.*, **16**: 42, 1937.
2. (To a book)—Doe, J. A., *The Dental Practice* (Chicago, Smith: 1930), p. 114. See McBride, *J. D. Res.*, **16**: 537, 1937.

Papers presented at society meetings should be so footnoted. Reprints, in any number, will be supplied at prices quoted on request and must be ordered at the time author's proof is returned.

Subscription price for the volume (issued bimonthly beginning with February) is $5.00. Single copies $1.00.

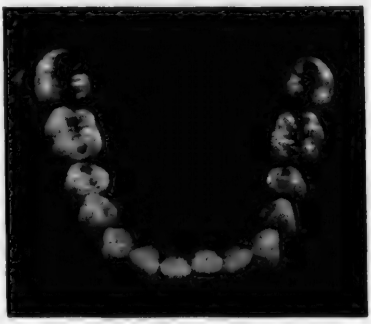
Address all communications relating to editorial matters to the JOURNAL OF DENTAL RESEARCH, 4559 Scott Ave., St. Louis, Mo.; advertising or business matters to the JOURNAL OF DENTAL RESEARCH, 12th and Clay Sts., Richmond, Va.; current circulation matters to the JOURNAL OF DENTAL RESEARCH, Greene and Lombard Sts., Baltimore, Md.; matters relating to Volume I–XV (1919–1936) to the JOURNAL OF DENTAL RESEARCH, 632 West 168th St., New York City.

TRUBYTE POSTERIORS

Practice-Builders! Why? Because they give Patients Comfort and Chewing Efficiency. How?

By Scientific Duplication of the Functions of Natural Teeth

WHEN patients are young, natural posteriors present high, sharp cusps with deep fossae and sulci, usually with a bite too deep for artificial teeth.



BY MIDDLE life, articulating facets have been worn on the cusps of both sets, the bite is more shallow and lateral trauma is reduced. This is the stage of high efficiency in mastication. It is reproduced in regular Trubyte Posteriors, which are suitable for firm ridges and careful articulation.

NEW Trubyte 20° Posteriors reproduce an engineering adaptation to considerably worn natural posteriors. They make stability and ease of mastication possible in flat and flabby ridge cases.

Trubyte Posteriors Meet All Your Requirements

THE DENTISTS' SUPPLY COMPANY OF NEW YORK

VITALLIUM *Scores Again!*

EXCELS ALL OTHER METALS

in Orthopedic Surgery

•

The matchless properties of Vitallium for prosthetics prompted its use for the fixation of fractures. It is the only true Cobalt-Chromium alloy being used in Dentistry and Surgery

There is a Vitallium Distributor near you

TRADE MARK VITALLIUM REG. U.S. PAT. OFF.

AUSTENAL LABORATORIES, INC.

34 West 33rd Street
New York

•

5932 Wentworth Avenue
Chicago

BRAUER, HIGLEY AND BOYD

DENTISTRY FOR CHILDREN

THIS new book considers the many problems of the child patient as they concern the dentist in his daily practice. It presents the subject lucidly and comprehensively, bringing together material from several specialized fields in a practical manner so as to be of genuine value to both the general dentist and the specialist. There are useful charts and tables and 335 excellent illustrations. It is written by an eminent Pedodontist, with contributions by a highly recognized Orthodontist and an outstanding Pediatrician.

335 Illus. 373 Pages. Washable Fabric **$6.50**

By JOHN CHARLES BRAUER, D.D.S., F.A.C.D., *Head of Dept. of Preventive Dentistry, Prof. of Pedodontics, and Director of the Bureau of Dental Hygiene, University of Iowa, School of Dentistry. With Special Chapters By* L. B. HIGLEY, D.D.S., F.A.C.D *Professor and Head of The Dept. of Orthodontics, University of Iowa, School of Dentistry; and* JULIAN D. BOYD, M.D. *Assoc. Professor of Pediatrics, College of Medicine, University of Iowa.*

THE BLAKISTON COMPANY 1012 Walnut St., Philadelphia

SEND FOR YOUR COPY!

THE 1939

Illustrated

PRICE LIST

of

COLUMBIA DENTOFORMS

Showing new and interesting developments in demonstration models.

Write name and address here

Dr .....

Address .....

City .....

and Mail to—

COLUMBIA DENTOFORM CORP.

"The House of a Thousand Models"

131 EAST 23rd STREET NEW YORK, N. Y.

PERFORMANCE TESTED

Under actual mouth conditions, DURODENT-ENAMEL, the non-leaking translucent plastic porcelain, has no peer! Here's why. . . . . Although it's denser, it has life-like translucency, will not check, crack or become chalky and its setting time is practically uniform under all temperatures. Try it soon!

OSKAR SCHAEFER

Manufacturer Chemical Dental Products

1 Wilkinson Ave., Jersey City, N. J.

Journal of Dental Research
Lombard and Greene Sts.,
Baltimore, Md.

Please send The Journal to me to the address given below, beginning with the.....issue.

Name.....

Street .....

City.....

.....

(In writing to advertisers, please mention the journal—it helps.)

THERE IS ONLY ONE

ARISTALOY

IT CANNOT BE IMITATED

ARISTALOY'S smooth particles insure a filling that will stay bright; it's controlled expansion within 8 to 10 microns prevent danger of the filling rising from the cavity. Aristaloy is easier to mix, easier to carve than amalgams containing irregular chips. There are four combination packages that offer worth while savings. Ask your dealer about them.

BAKER & CO., INC.

113 Astor St., Newark, N. J.

NEW YORK SAN FRANCISCO CHICAGO

The following advertisements are of dentifrice manufacturers who are cooperating with the Council on Dental Therapeutics of the American Dental Association in fostering rational therapeutics and truthful advertising.

These dentifrices have been investigated and were awarded the A. D. A. seal of acceptance.

They warrant your patronage.

DETROIT DENTAL AID COMMISSION **Dental Aid Tooth Powder** **Detroit District Dental Society** **Detroit, Michigan**	**LACTONA DENTIFRICE** **Lactona, Incorporated** **St. Paul,** **Minnesota**
SHEFFIELD'S TOOTH PASTE **The Sheffield Company** **New London, Conn.** **3132 S. Canal St., Chicago, Ill.** **500 Fifth Ave., New York, N. Y.**	**McCANN'S DENTAL POWDER** **McCann Dental Powder Company** **Danville,** **Illinois**
PRO-PHY-LAC-TIC BRAND TOOTH POWDER **Prophylactic Brush Company** **Florence, Massachusetts**	**PYCOPÉ TOOTH POWDER** **Pycopé Incorporated** **2 High Street** **Jersey City, N. J.**

Appreciation

The International Association for Dental Research, and all of its representatives in the Board of Editors of the Journal of Dental Research, hereby express their hearty appreciation of the financial support of the advertisers in this issue of the Journal. We commend these firms to the consideration of all who are interested in the advancement of dental research.

JOURNAL OF DENTAL RESEARCH